THE EFFECTS OF AGE UPON CALCIUM ABSORPTION, DEPOSITION AND EXCHANGE IN ANIMALS

By

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INTRODUCTION

The fundamental role of calcium in the nutrition of animals is well recognized, and the physiological and chemical behavior as affected by dietary, endocrine and various stress factors has been widely investigated. However, there are controversal opinions concerning several of these complex relationships, and particularly the nutritional aspects of calcium metabolism that warrant further study.

The function of the blood as a common carrier of metabolites, and as the initial pool for calcium should permit it to reflect the simultaneous metabolic activity of the entire body. Studies of blood calcium concentration, however, give no indication of the rate of flow or the direction, whether into excretory channels or into the tissue. A measure of the variable rate of excretion could be used to determine the influence of many factors upon calcium metabolism, and concurrent studies indicating the destination of the element would provide basic information on the fundamental metabolic processes involved.

There is no complete agreement upon the factors nor the process involved in the laydown of calcium in bone. Only recently has it been possible by tracer techniques to differentiate between calcium deposited and that removed from the bone (49). However, the picture is still complicated by the fact that the element may be removed from the bone inorganic tissue by either surface exchange and/or dissolution by some means of the bone salt crystal. Likewise, the metabolic behavior of the element in bone has been obscured by the complexity of the absorption and exchange mechanism, as well as the dietary influences.

With our present knowledge, it is not clear whether the effects of age upon calcium behavior in the animal body is being partially masked by the reciprocal influence of calcium upon the ageing process. Ageing has been associated with the accumulation of calcium, especially during the senescent period, in the cells (73, 74) and in certain body tissues. In specific pathological investigations with tumors and cancerous tissues and in longevity studies there has been noted an apparent shift of calcium from the bones to the soft tissues (58, 73) resulting in bone fragility, fractures, metastatic calcification, arteriosclerosis, cataracts and other malfunctions associated with calcification. It is obviously difficult to determine whether these changes with increasing age are the cause or the consequence of senescense.

In the young animal the lack of a means for the distinct separation of age from growth influences has complicated the study and impeded the interpretation of the data in the literature germane to the immediate age effects upon calcium metabolism. Likewise, it is not quite clear whether growth itself is due to a higher rate of calcium accretion or to a lower rate of degradation. Sherman (114) has proposed that calcium accretion is not always related to growth and that normal growth rate, as expressed by the body weight at a given age, "is in itself not a disturbing factor" in the rate of calcification (17). Therefore, if it is assumed that the requirement for dietary calcium in a growing animal is not augmented to any appreciable extent by maintenance requirements (62, 110), age might well be considered as the chief factor responsible for the increasing percentage of calcium in the animal body.

Factors that govern the absorption of calcium from the gastrointestinal tract are vaguely understood, and there is no complete agreement on how or in what state the element crosses the intestinal membranes (63). It has been generally recognized that as an animal grows older there is less efficient utilization of ingested calcium. However, there has been less understanding of the requirements for calcium after growth ceases. True digestibility in mineral balance studies is difficult to estimate because of the lack of adequate means of differentiation between the endogenous and exogenous fractions. It is not clear, therefore, whether this decreased efficiency with age is due to poor absorption from the tract, or poor retention by the tissues or both.

Recent advances in radioisotope procedures have made possible the separation of some of these various aspects of calcium metabolism due to the physiological changes associated with growth, ageing and senility. Isotope techniques are unique in that they offer the possibility of measuring the individual rates of opposing reactions within the tissue or the animal body itself, and permit the application of the newer concept of dynamic equilibrium to the study of calcium behavior. The fundamental concept of tracer studies is that these tracer elements possess the same chemical properties and are treated by organisms the same as the naturally occurring stable isotope of that element, while having a distinctive physical property that is subject to measurement.

It has been the purpose of this investigation to study the effects of age upon calcium behavior in the bovine and the rat under controlled conditions with the use of radiocalcium. Although this study was carried out with normal animals of definite chronological ages under laboratory conditions, for clarity of discussion and correlation with the literature, consideration will be given to four arbitrary physiological periods of development (1) embryonic--from conception to parturition (2) growth--from parturition to puberty (3) maturity--from sexual maturity to twice puberty and (4) senescent--the latter one-fourth of the expected life span for the species. The objectives of the study have been: (1) to determine the rates and pathway of calcium excreted as affected by age in the two species. (2) To estimate the calcium absorption and the partition of the excreted fecal calcium into the endogenous and exogenous fractions. (3) To measure the total calcium and the deposition of labeled calcium in selected tissues of animals at various ages and stages of development. (4) To correlate the total and labeled calcium in the blood and various tissues of these species in an effort to determine the degree of exchange associated with the tissue metabolic activity at the different ages. (5) To demonstrate by means of autoradiograms the deposition pattern of calcium in the long bones of the animals at different ages for correlation with the chemical and isotope analysis. (6) To study any changes in the calcium to phosphorus and calcium to ash ratios in the tissues of the various age groups and (7) to interpret the results of the concurrent chemical and radioisotope studies in terms of the overall calcium exchange potential at the ages indicated.

REVIEW OF LITERATURE

Basic Considerations

Age and whole body composition: The fundamental concepts of ageing have been formulated from data compiled from chemical composition studies on the whole animal body at the different stages of development and at maturity. Weights, measurements and elemental analysis of entire bodies of experimental animals were included in many of these investigations. It was thus early established that marked changes occurred in the percentages of water, fat, nitrogen and ash from conception until death. Mitchell *et al.* (94) and Mitchell and Hamilton (96) have described these changes in chemical composition of the pig from the fifth week of gestation to maturity. Swanson (124) reported separate analysis for the whole bodies of thirty six individual pigs ranging in weight from twenty to 400 pounds. Moulton *et al.* (97, 98) reported the values for water, ash and protein composition of the ox and man from early embryonic age to senescense and established the stages of "chemical maturity" for these species.

Haecker (39) has recorded separate analysis for the whole bodies of forty nine individual cattle ranging in weight from 100 to 1,500 pounds. Recently Ellenberger *et al.* (31) have presented a detailed report on the chemical composition of the whole bodies of dairy cattle with increasing ages. Hatai (54) and Chautin (21) analyzed rats at various stages of growth from birth to maturity for water, protein and fat. There is general agreement, therefore, that during the early period of development skeletal growth rate exceeds that of the body as a whole, and that on a percentage basis, water decreases, and dry matter, ash and fat increase with increasing age of the animal. No significant changes, associated with age increase, have been noted in blood, ligaments or tendons and muscles, and body fluids show no clearly defined age change except dehydration (126). The fetus increases in weight mainly during the last quarter of pregnancy, with the greatest percentage of increase in ash and dry matter occurring during the last trimester of pregnancy (117).

Age and total body calcium: The basic information obtained from whole carcass analysis has added much to the knowledge of the role of calcium in the structure, growth and function of the animal body and is relevant to a discussion on age influences. During the period from conception to senesence a general increase in total body calcium, as well as average per cent of body calcium has been noted with advancing age in rats (18, 27, 54, 81, 104, 115), swine (61, 90, 94, 96, 122) and cattle (31, 39, 97, 99). Negligible amounts of calcium are deposited in the fetus before the last trimester of pregnancy, and over sixty per cent of the skeletal calcium of the newborn is the result of deposition during the last one fourth of the prenatal period (18, 117).

In the normal, growing rat Sherman and MacLeod (115) found the per cent of calcium in the body to increase from 0.25 per cent at birth to 0.6 per cent at fifteen days, 0.7 per cent at thirty days, 0.75 per cent at sixty days, 0.95 per cent at ninety days and 1.2 per cent at maturity. Total calcium and per cent of the element increased rapidly up to ninety days and then at a slower rate to adult age. Spray and Widdowson (122) reported a large increase in calcium concentration to take place in the rat and the pig during the suckling period, after which the rate of accretion fell gradually until the percentage characteristic of maturity was reached. In the rat this period of "chemical maturity" was at 100 days and in the pig at 120 days as compared to seven months for the bovine (98). In a study of the phosphorus content of the body in relation to age, growth and food, Sherman and Quinn (116) found that during growth from birth to 459 days, the body phosphorus increased in a greater ratio than the body weight, but not in such a great ratio as did the body calcium. The average adult male had seventy times the body weight with which he was born, but 150 times as much phosphorus and 340 times as much calcium as did the body at birth. During the first fifteen days of life the average rat increased his body weight five times, body phosphorus seven times and his body calcium twelve times. In a report of the calcium content of the normal growing body at a given age, Briwa and Sherman (17) postulated that age was the "predominant determining factor" in the increase percent of calcium in the body of the rat.

A comprehensive study of the whole bodies of 132 dairy animals from 135 day fetus to twelve year old cows by Ellenberger _et al._ (31) showed whole body calcium and skeletal calcium to increase steadily from prenatal life to maturity. On a fat free, dry matter basis, the percentage of calcium in the skeletons increased from 12.4 to 14.8 per cent in the fetus groups, and from fourteen to 18.3 per cent from birth to twelve months of age, and upward to 21.8 per cent for the forty nine mature cows. A corresponding increase was noted for the inorganic phosphorus. Complete mineral analysis of fifteen calves and eighteen steers from birth to four years of age by Hogan and Nierman (28) indicated an increase of calcium, phosphorus and magnesium with increasing age.

It has been observed by several investigators that calcium reaches a higher concentration in the bodies of females than of male rats. Sherman and MacLeod (115), Duel et al. (30), Spray and Widdowson (122) and Cox and Imboden (27) have reported increased calcium in females over males from carcass analysis. Likewise, Zucker and Zucker (133) noted better calcification in female rat femurs, and Hammett (40) reported that the humerus and femurs of the female contained a higher proportion of ash than did the corresponding bones of the male from the age of twenty three days. There was a tendency toward a fixed composition in the male at sixty five days and in the females at 100 days. However, Fairbanks and Mitchell (33) have attributed the greater percentage of calcium commonly reported in the bodies and bones of females to be "entirely referrable to their slower growth". Recently Kim et al. (69) have attributed this difference in growth rate to the sex hormones. Feeding isocaloric diets to rats they found that the males showed a greater and more rapid growth than the females even when fed equal amounts of the same foods. Whereas testosterone treated females showed a greater increase in weight than the paired controls.

Age and Calcium Absorption

Calcium balance studies: It has been demonstrated repeatedly that as age increases, an increased intake of calcium and phosphorus is required for calcium balance in man (62, 73, 117), rats (18, 27, 33, 57, 58, 62), swine (61, 90, 91, 96), and in cattle (90, 93, 100, 131). Comprehensive reviews concerned with the absorption, excretion and the function of calcium in animals are to be found in the literature (9, 32, 38, 62, 91, 95, 113, 114, 123).

Kane and McCay (66) compared old and young growing animals in their ability to absorb dietary calcium incorporated into complete diets at levels of 0.3, 0.5 and 0.7 per cent calcium and 0.4 per cent phosphorus. The forty to fifty day old rats stored seventy eight to eighty eight per cent of the calcium from such diets during growth, while the 600 day old animals could not even maintain their body stores at the lower level, but were in equilibrium when 0.5 per cent calcium was fed. Further increase up to 0.7 per cent calcium did not give increased retention. This tendency to go into negative calcium balance started about the beginning of the last one third of the life span. McCay et al. (85) reported that rats stored from a diet containing 0.3 per cent calcium, ninety per cent at thirty eight to 110 days of age while at 160 days of age only fifty to sixty five per cent of the calcium was retained.

Balance studies with young rats on a minimum dietary calcium intake indicated that there was a change from a positive balance at thirty days of age to a negative balance at six months and older (58). Older animals have been found to be less sensitive to changes in dietary phosphorus levels, as far as calcium behavior was concerned. Henry and Kon (57) reported a decreased calcium absorption regardless of diet concentration as the age of the animal increased from one month to two years. On a diet containing 0.13 per cent of calcium and 0.22 per cent phosphorus they found that the absorption of calcium from the diet between the ages of four to eight weeks was ninety six per cent; twenty to thirty weeks, twenty five per cent; fifty six to sixty weeks, fifteen per cent; and, at the age of ninety three to ninety seven weeks the animals were in negative balance. In another group of animals, 102 weeks of age, 0.46 per cent calcium and 0.60 per cent phosphorus were required to establish equilibrium.

Bloom (14) reported ninety three per cent retention of calcium with rats thirty three days of age from a diet containing 0.45 per cent calcium and 0.34 per cent phosphorus, but at sixty three days these animals retained only fifty eight per cent of the calcium for equilibrium.

Nicolayson (103) reported the absorption of calcium, as measured in intestinal loops and in the intact rat, to be higher in the young than old animals. He postulated calcium absorption to be dependent upon both growth and age. Hegsted et al. (56) confirmed these findings and concluded that there is no evidence that calcium deficiency occurred in adult males. He postulated the urinary calcium excretion to show a general excellent correlation with the calcium intake level.

Lindsey et al. (78) made the interesting finding that in dairy cattle the intake per unit of body weight of both calcium and phosphorus decreased with increasing age, and retention per unit of weight nearly always decreased in a similar manner.

In man Knapp (68) found from an extensive study with 600 normal persons from one to eighty years of age that the mean urinary calcium excretion increased with age. He thus considered the quantity of urinary excretion to be dependent upon an endogenous factor.

Sherman (114) has expressed the opinion that the retention of calcium in young rats is not always related to growth. Balance experiments with both rats and children have indicated that increased retention of nitrogen was not always accompanied by increased retention of calcium. This has been confirmed by Minnesota workers with rats (128), and demonstrates that within the periods of normal growth, neither larger size as an individual characteristic, nor larger size induced by higher protein feeding, is associated with correspondingly advanced skeletal development as reflected by whole body or skeletal calcium retention. Thus it is apparent that calcium behavior is more closely related to age than to body weight.

Radiocalcium balance studies: It has long been recognized that equilibrium or positive balance is not necessarily proof of adequate uptake, but may be a reflection of the level of calcium previously received (47, 95, 110). Admittedly balance measurements of calcium absorption are in error to the extent of the proportion of the calcium in the feces representing metabolic exchange (113, 42). That is, the fecal calcium includes not only the unabsorbed calcium from the feed but also calcium that has been absorbed and re-excreted and exchanged bone calcium that is being eliminated as an endogenous fraction, (49, 90). Isotope techniques have been employed for the quantitative separation of these fractions by Hansard _et al._ in the steer (42, 48, 49) and in rats (51) by the integration of concurrent chemical and radiocalcium balance studies. Steers on normal and high calcium rations excreted directly without absorption fifty four and seventy per cent, respectively, of total calcium ingested while seven and four per cent thereof was absorbed and re-excreted. It was calculated that nine and five grams, respectively, of endogenous calcium was in the feces from animals receiving normal and high dietary calcium intakes. A direct determination of endogenous fecal calcium by isotope dilution by Visek _et al._ (127) supports this procedure and demonstrates a means of estimating "true digestibility" as influenced by certain physiological and nutritional factors. These methods give no information as to the pathway by which the endogenous calcium reaches the feces, but do indicate an overall measure.

There have been no extensive studies reporting the behavior of radiocalcium with animals of different ages. However, in a comprehensive study with calcium 45 by Carlsson (19) young growing rats were reported to retain twenty per cent more of an oral dose than did mature animals. The absorption and retention in the body as a whole, and in certain individual bones was found to be much lower in rats 210 days old than at seventy days of age. No relationship was noted between calcium 45 absorption and the rate of growth. However, on limited dietary calcium intake, increased absorption was shown to be dependent to some extent upon the rate of animal growth. Hansard et al. (47) reported that weanling rats on a normal synthetic diet retained seventy seven per cent of an oral dose of radiocalcium as compared with sixty seven and fifty per cent, respectively, for rats ninety and 180 days of age. Little difference was noted for rats at six months and sixteen months of age. Fecal excretion of orally administered calcium 45 was postulated to be an extremely sensitive indication of the relative calcium status of animals (46).

Copp (26) reported the retention of radiocalcium to be seventy three per cent in young normal rats; however, retention was only thirty two per cent in the normal adults. In rachitic rats there was an initial rapid uptake of calcium by the skeleton, but was rapidly released indicating a labile combination with bone, possibly due to exchange with labile calcium on the surface of the bone salt crystals.

Age and Blood Calcium

Blood not only serves in the transport of calcium but is the primary metabolic pool between the gastro-intestinal tract and the soft tissues and bones. This function as a common carrier permits it to reflect simultaneous metabolic activity of the whole animal body, and a study of the kinetics is basic in the understanding of the physiological functions of any metabolite. The concentration, state and function of calcium in the blood of the various animal species has been reviewed by the several investigators (79, 87, 90, 113).

The physiological mechanisms of the body tend to maintain the constancy of the concentration of all circulating minerals of the blood; some more than others and calcium is rigidly controlled (95). Thus, there is a balance between absorption, excretion, deposition and mobilization. Therefore, either calcification or decalcification may take place without great change in blood calcium concentration (8). Calcium may leave the system by way of the feces or urine or it may go into the bone. The factors which govern the absorption of calcium into the blood stream and those which control its removal act in unison. Thus the blood may draw its calcium from either the food ingested or from the stores in the skeletal trabeculae as it circulates through the body (61) and a dynamic equilibrium is maintained between the serum and skeletal calcium (33, 53, 79, 87).

The relationship of serum calcium to the several storehouses for the element is of special interest as associated with the concepts of the state of calcium in the blood and those of bone mineralization. The process of calcification is dependent ultimately upon the calcium and the phosphorus in the blood, whatever may be the determinant of precipitation of the mineral in the bone (117).

Blood calcium has been shown to be generally independent of the age of the animal. However, the general findings seem to indicate that both serum calcium and inorganic phosphorus levels in young and fetal animals are slightly higher than in the mature adult.

Huggett (64) reported an increased blood serum calcium value in rats and man with increased age. Malon (88) with sheep and Green and Macaskill (37) with calves, found total and inorganic phosphorus about twice as high in the fetal as maternal blood, but no apparent changes in the blood calcium levels, except for a temporary increase following parturition. Sato (112) reported the calcium content of the fetal venous blood serum to be slightly higher than that of the arterial serum, and considerably higher than the calcium of the maternal serum of the cow. Likewise Plumlee *et al.* (105, 107) demonstrated fetal blood calcium values to be higher than those of the maternal blood. Ellenberger (31) noted no great change in blood composition from calfhood to maturity except for a higher ash and calcium value for the very young animals. Robertson (109) reported the blood calcium and phosphorus content of aged normal adults not to differ significantly from those of young men, noting only a slightly higher phosphatase level in the adults.

Age and Calcium Deposition and Exchange

General considerations: The skeleton functions both as a structural organ and as a storehouse for more than ninety eight per cent of the body calcium. It is generally accepted that bone is a vascular structure and that the minerals may be readily removed and transferred to the circulating body fluids (8, 79). There is virtually unanimity to the effect that the fundamental crystal structure of the bone salt is an apatite of which the prototype is fluorapatite; that this salt is not of definite chemical composition, but that changes and substitutions continue to occur long after calcium is deposited (8). The "admixture of apatite and carbonate" (86) in bone is in a dynamic state (20) and provides a means of ready exchange from or into the accessible metabolic pools.

Maximow and Bloom (89) discuss the internal reconstruction of growing bone as a "constantly changing process" taking place throughout the entire period of development and even to adult age. This consists of the formation of new lamellae by the osteoblasts, with simultaneous destruction and digestion of the recently formed "areas of bone tissue" and the consequent formation of new layers. The amount of calcium ultimately remaining in the bone is the difference between the amount deposited as a consequence of bone salt precipitation in the growing matrix and the amount removed by bone dissolution (79). Whether this relationship between skeletal deposition and retention is dependent upon the precipitation or upon the dissolution, or both, has not been clearly established (20).

Bell *et al.* (11) have made a quantitative approach to this problem in a study of bone size, as well as certain chemical and physical properties of the femur of young rats fed diets of varying calcium content. The main effect of calcium starvation was found to be an increase in the width of the marrow cavity, while the outer dimension of the bone remained practically unaffected. They postulated, therefore, that calcium for bone formation was resorbed from the walls of the medullary cavity or that it was supplied by the food.

Bauer *et al.* (8) have demonstrated with rats that during the growth period, or during negative calcium balance periods, the trabeculae of bones were easily depleted. Likewise during positive balances, the calcium was deposited readily in these lace-like trabeculae located near the ends of the bones. In the adult animal, the easily mobilized calcium was also deposited in the trabeculae and not in the bone shaft (89). This has been confirmed by other investigators, in essence if not in order of magnitude (46, 48).

Theories of calcium deposition in bone have been reviewed by Reed (108), Logan (79) and Weinman *et al.* (132), and the concepts of decalcification have been discussed by McLean (86) and Nicolaysen (103). There is apparently no complete agreement, however, on the processes nor the factors involved. The cells specific to osseous tissue include osteoblasts, osteocytes and osteoclasts associated respectively with the formation, the maintenance and the destruction of bone, but the exact relationship of the cells to these processes has remained largely obscure. In consideration of the major theories relative to the process of deposition it is emphasized that none of these explain adequately the whole phenomenon of calcification. The concept of decalcification of bone is that the bone salt is broken down by some active principle, the nature of which is not clear, which at the same time destroys the organic matrix (86).

Calcium deposition in bones: Calcium metabolism of the bones involves not only the deposition during growth, but also processes of storage and mobilization throughout the life of the animal (90, 117). Because of the great percentage of the body calcium in the bone and its apparent availability for use by the various metabolic pools, it is not surprising that this tissue would be more sensitive to age changes than other parts of the animal body.

The teeth have long been used as an indicator of age in farm animals. The early work of Hudekoper (65) can still be consulted with profit since it reveals the changes with age of the various species including swine and dogs. The teeth of rats, subjected to varying rates of growth and at ages from 474 to 1369 days, have been examined by Smith and McCay (121). In contrast to the slowing down of deterioriation in other organs of rats that were retarded in growth, teeth decay appeared to be augmented more rapidly at an early age. These constant and relatively slow changes with age have caused the teeth to be used by many investigators as biological reference standards for comparative studies between tissues in the body (77, 85, 121).

In a study of the chemical differentiation of bones during growth, Hammett (40) compared the composition of the humerus and femur of normal rats from twenty three to 150 days of age. He reported a marked drop in growth capacity at thirty days and again at sixty five days of age. The first change he attributed to feed adaptation and the second to puberty. The degree of ossification of the humerus was found to be greater than that of the femur.

Becks et al. (10) studied the changes in the proximal tibial epiphysis of rats ranging in age from five to 546 days. He reported the cartilage to decrease in width quite abruptly at seventy four to 123 days of age, which coincides with the period of decreased growth rate. With advancing age, the amount of enclosed cartilage within the trabeculae decreased and the trabeculae themselves were reduced in number. This indicated a probable change with age in the pattern of bony deposition. Eventually the cartilage plates became almost completely sealed off from the marrow and thus from encroachment of blood vessels.

In studies on the affect of retarded growth upon the length of life span McCay (82) observed that the aged bones of retarded rats were all demineralized despite the adequate rations they were careful to supply. There are yet many problems, therefore, associated with the changing needs of the ageing animal for the several nutritionals.

Other investigators previously referred to (31, 54, 115, 122, 133) have studied the entire skeleton changes and calcium accretion with increasing age. It has been generally concluded that age predominates over growth insofar as calcium accretion is concerned (114, 133) and is therefore the principal factor responsible for the increased percentage of calcium in the animal body.

Calcium exchange in bones: Tracer techniques have made it possible to differentiate with some degree of accuracy between calcium deposited and that removed from the bone (48, 49). However, the true significance of this procedure has been partially masked by the concepts of recrystallization and surface exchange (8, 34, 101, 102, 120). The fundamental role of the physical phenomenon of exchange is based on the fact that calcium ions in the plasma which are in contact with bone surfaces may readily exchange with calcium ions located in the surface of the bone crystals. This exchange is rapid and does not in itself result in the accretion of bone (53, 59, 60). In vivo studies (46, 50, 51, 52) have indicated that when calcium 45 ions are introduced into the circulating blood either through the vein or via absorption from the gastro-intestinal tract, they disappear rapidly from the blood to the soft tissue and bones until the ratio of labeled calcium to total calcium (specific activity) in the blood and soft tissues reach an equal value. Eventually a like equilibrium is reached between the plasma and certain portions of the bone, although the concentration of calcium 45 in the bone, due to its higher total calcium content, may be several hundred times that of the plasma. This has been interpreted as indicating that these calcium ions are readily exchangeable, and these specific activity values have been employed to demonstrate the rate of calcium turnover or exchange in the various tissues (24, 46, 48, 105, 119). It has been proposed that these values may be sensitive indicators of the metabolic activity of the bones or soft tissues (47, 106).

In vitro studies (1, 4, 34, 60, 101, 102) have verified these findings and demonstrated feasible means for the study of the rate and magnitude of bone absorption and exchange. Falkenheim *et al.* (34) reported that fresh bone was found to take up labeled calcium in a continuous process that may involve both exchange and recrystallization, and that as much as twenty per cent of the bone calcium entered into this exchange reaction. Copp *et al.* (26), and Singer and Armstrong (119, 120) reported in rats the existance of a "mobile" and "fixed" fraction of skeletal calcium with marked different rates of exchange which they theorized as due to the fact that the bone salts exchange calcium on the crystal surfaces rapidly and that exchange with that on the interior of crystals takes place more slowly by recrystallization. Neuman and Mulryan (102) suggest that the rate of crystallization decreases with age of the animal.

Autoradiographic techniques have been developed that permit visualization of the pattern of laydown of calcium in the gross anatomical areas of bone and offer a method for further study of the factors governing deposition and removal (5, 24, 25, 80). Regions of periosteal origin have been thus characterized by sharp images, relatively slow deposition and slow removal; and, regions of endochrondral origin by diffuse images, rapid deposition and rapid removal (24).

Several investigations are reported in the literature whereby the application of these unique procedures have been applied to the study of age and calcium metabolism in bone.

In a comparison of turnover rate in mature teeth of rats fed radiocalcium and radiophosphorus, Armstrong and Barnum (2) found that the rate of exchange of the radioactive ions in the molar dentine was about one fourth that of the femur, while that of the incisors was two thirds that of the femur. Harrison and Harrison (53) reported the turnover rate of labeled calcium in the bones of the rat to decrease with increasing age and proposed that this may be due to differences in bone structure, or to decreased metabolic activity, or to both. Hansard et al. (51) considered the rate of uptake and the exchange to vary directly with the metabolic activity of the tissue involved. This was verified by Carlsson (19) who found no significant differences in the uptake or turnover rate of calcium 45 in the incisors of seventy and 210 day old rats, but a marked decrease in exchange of femurs with increasing age.

Further age studies (47) have verified the progressive increase of total calcium and ash content of the femur bones in rats from one to sixteen months old and demonstrated calcium 45 accumulation, and turnover rate to show a progressive decrease with increasing age. There were indications that after six months the capacity for calcium accumulation in the bone from extracellular fluids had decreased, and it was suggested that the bone concentration of calcium 45 per unit of calcium 45 absorbed may be a sensitive indication of the exchange potential of the bone.

Plumlee et al. (105, 106) in a study with pregnant heifers sacrificed seven days following an oral dose of calcium 45, reported uptake and turnover of calcium in the fetal bones to exceed that in the bones of the dam. There was a decrease in specific activity of all bones with increasing fetal age. The pattern of calcium 45 laydown was demonstrated by autoradiographs to substantiate the radiochemical data. It was evidenced from these findings that the rate of calcium exchange was a function of age and that there was a marked tendency for this rate to decrease with increasing fetal age depending upon: (1) the calcifying activity of the bone, (2) the exchange capacity of the calcifying area, (3) the amount of contact between the circulating blood plasma or other metabolic pools and the bone crystals, and (4) the increasing amount of stable calcium deposited in the bone.

Calcium deposition in soft tissue: The one per cent of calcium not found in the skeleton is widely distributed in the body tissues and fluids, and is of great physiological importance. Radioisotope studies have indicated that the calcium of the blood and soft tissue are in equilibrium (50, 53, 79, 107) and are thus maintained by skeletal influences and physico-chemical properties of the calcium-protein compounds of the blood. It is generally accepted that calcium is closely associated with the physiologically active tissues of the body, and that exchange is closely related to their metabolic activity (12, 19, 47, 49, 105, 114). It is to be expected, therefore, that the calcium content of the various organs will be related to their stage of development and rate of growth (54, 64).

Brody (15, 16) has proposed that the placental growth of rats is divided into a number of periods, each being distinguished by its particular constant percentage weight increase. Kirslovsky and Larchin

(70) have postulated four such periods in the prenatal growth of cattle. Barcroft (6), however, suggested that for different organs of the developing fetal body there are critical periods during gestation at which time they severally show a maximum weight increase due to growth, and that they do not occur simultaneously. Wallace (129) reported that the sheep fetal organs, such as kidney, liver, lungs and brain, make a larger proportion of their fetal growth during the early stages than do such organs as spleen, thymus, intestines and skeleton. In consideration of fetal composition, therefore, age of particular organs as reflected by the stage of their development would influence their calcium content. Under conditions of restricted calcium nutrition, nerve tissue competes for available nutrients more effectively than do soft tissues and more effectively than bone. Sherman _et al._ (116) has reported this to be especially true for phosphorus in young growing animals.

In a review of the physiological aspects of ageing, Lansing (72, 73) has discussed the important and intricate mechanisms involved in soft tissue calcium deposition. Calcium increase in soft tissue appeared to be more a cause than an effect of the ageing process.

Previous to the introduction of radiocalcium into the study of tissue metabolism, calcium laydown effects could not be measured in tissue except by differences in accumulative amounts. Small differences were therefore difficult to estimate by chemical analysis of the soft tissues, and conclusions from the literature reporting accretion as an ageing effect are obscure. The tissue changes accompanying senescence in man seem to involve rather generally an increase in calcium content

(117) particularly in tissues such as the hyaline cartilage. Sclerosis of the large arteries involve deposition of calcium salts similar in composition to bone salts (92), while deposition of calcium phosphate in the soft tissues accompanies many types of senile degeneration and disease associated with old age (20, 36, 71, 83). Frondel and Prien (36) have, by chemical, optical and X-ray studies, identified carbonate apatite as the principle inorganic salt in these pathological calcifications of the aorta, in arterio-sclerosis and also as a common constituent of kidney and urinary bladder stones.

Hogan and Neuman (61), in analysis of cattle sacrificed at intervals from birth to four years of age, did not find the calcium content of the hide and hair, fat or soft tissue to be materially affected by age, or condition.

In the course of twenty years of research at Cornell (84) heavily calcified arteries and kidneys have been noted in many old rats, while in subsequent groups these tissues were relatively free of calcium deposits. This soft tissue calcification was accredited to an unknown variable in the diet and not to the dietary calcium itself.

Plumlee et al. (105) compared the calcium content of soft tissues from bovine fetuses three, six and eight months old with that of twenty four to thirty month old dams. He reported these tissues from the three month fetuses to exhibit a significantly higher calcium value than those from the dam or from the older fetuses.

Other investigators, however, have associated increased calcium values with progressing age in various species. In view of the postulated shift with age of calcium from bone to soft tissue after maturity (58, 73), and since calcium tends to form insoluble compounds, it would not be surprising to find that this physiologically important element did tend to accumulate with increasing age. Increases of calcium with age have been reported for the brain of the guinea pig, sclera of the cat, arteries, kidneys, heart and a variety of other human tissues. Lansing et al. (74) noted a parallel increase in the elastic fibers and calcium deposition with age in the human left coronary artery. There were indications that this calcium increase paralleled a decrease in magnesium, but this observation was not confirmed. In further studies with malignant tissues, Lansing (71) reported ageing to be associated with an increase in calcium content of the tissue, but diametrically opposed to this pattern was the observation that in active cancerous tissue there was a decrease in total calcium. The mechanism responsible for calcium uptake was postulated as involving a protein as its physical basis and occurring in the cortex of the cell.

Barnes (7) reported calcification of the auricle of the heart and calcium deposition in kidneys as a function of age. Rats retarded in growth by limited caloric intake showed less calcification.

In a comparison of tissues from mature man and rats with those of senescent animals, Simms and Stolman (118) found the old tissues to contain more calcium, water and sodium and less nitrogen, phosphorus, magnesium, potassium and ash than corresponding tissues from mature individuals. This may indicate that the proposed shift, with age, of calcium from bone to soft tissue (58, 73) begins in a small way at the time of chemical maturity (97, 98, 122) and progressively increases with age.

Calcium exchange in soft tissues: It has been proposed that soft tissue calcium remains in equilibrium with blood and bone calcium by exchange (46, 50, 105) and that the per cent of exchangeable calcium was much higher in blood and soft tissue than in bone (53, 107). Furthermore, this exchangeable calcium was higher in the more active portion of the various skeletal tissues. These facts have been demonstrated by the use of radiocalcium, both *in vivo* (19, 46, 50) and *in vitro* (1, 34, 60). The principles and problems of the exchange concept have been discussed by the several investigators (26, 53, 59, 60), and is generally agreed that immediately after calcium 45 ions are introduced into the blood they are removed by the several competing processes such as mixing with body fluids and deposition in the soft tissues and bones, and excretion by the normal process of elimination. All these processes occur rapidly as indicated by the rapid attainment of equilibrium between tracer doses of radiocalcium with the calcium in the blood, soft tissues, and bone (25, 46, 105).

There are few reports in the literature concerning these laydown and exchange values as affected by age. However, in a detailed study of placental calcium transfer with fetuses of varying ages, Plumlee *et al.* (105, 107) measured the absorption and the exchange of radiocalcium within the various soft tissues at three, six and eight months gestation and reported no effect of age on these values for the fetus or those of the dam sacrificed 168 hours after oral administration of the isotope.

Lansing (75) administered radiocalcium intravenously to old and young mice and examined the livers for both free and bound calcium 45. His studies indicated that calcium in old livers was more firmly bound, as evidenced by the lower exchange rates. In young tissues the uptake was low, but the turnover rapid, while in the tissue of old animals the reverse was found. These turnover or exchange studies have thus been employed as a basis for estimating intracellular calcium changes as affected by age.

GENERAL EXPERIMENTAL PROCEDURE

Procedures with Animals

Selected cattle and rats of known breeding and nutritional background were used in this study. They were maintained on rations prior to and during the experimental period which were calculated to be adequate. An effort was made to reduce to a minimum, errors due to a difference in nutritional status, strain, individuality and other factors known to affect normal metabolic behavior. The study consisted of two distinct phases and for clarity of discussion, details of procedure for the two species and for the individual investigations will be presented separately.

Tracer levels of labeled calcium were used in these investigations to follow the metabolism of this element in animals of different ages. The radiocalcium was received as the chloride and was diluted, for administration, to an appropriate volume with distilled water. To prevent the possibility of precipitation upon standing, the pH was adjusted to 6.5 with sodium hydroxide or bicarbonate immediately before dosing.

The management of the animals previous to administration of the isotope and during the experimental period has been described in detail by Hansard (41, 44). In brief, following preparatory periods on known rations, the animals were oriented in metabolism units and were given a single dose of radiocalcium quantitatively (44, 45). The level of feeding was unchanged during the pre-experimental and experimental period. Feed intake was measured and feces and urine were collected at stated intervals of time after dosing for calcium and phosphorus balance studies and for radiocalcium excretion values. In the bovine, the blood calcium 45 picture was followed for 168 hours after oral and/or intravenous administration of the isotope. At sacrifice selected fresh tissue samples were weighed in tared crucibles for measurement of ash, total calcium and phosphorus and for radiocalcium determinations. For direct comparison all tissue distribution analysis data for labeled calcium were calculated on the same body weight basis for the species.

Methods of Analysis

In metabolism investigations involving labeled elements it is essential to have both the chemical and radioisotope measurements in order that specific activity may be calculated for the estimation of turnover rate. It was desirable therefore to integrate the chemical and radiocalcium analyses so as to minimize and simplify the steps involved for routine determinations on large numbers of samples. Details of this procedure have been described (23), but consist essentially of dry ashing the samples in a muffle furnace at 650° Centigrade, dissolving the ash in dilute hydrochloric acid, precipitating the calcium as the oxalate, drying under an infra-red lamp, weighing for mass and total calcium calculations and measuring the activity under a thin mica window Geiger-Muller counter tube in the usual manner.

Calcium 45 has been reported as having no gamma radiation, a maximum beta energy of 0.26 mev with a range of sixty four milligrams per square centimeter and a half life of 152 days (111). This relatively low beta energy and the consequent self absorption made it advantageous to have a minimum mass in the sample measured, and requires that suitable corrections be applied. It was necessary to add a standard amount of carrier calcium, usually about eight milligrams, to the blood plasma and the soft tissue ash previous to precipitation in the plastic tube and cup assembly for measurement of the activity. In all other samples, mass was kept below 200 milligrams of ash for soft tissues, and aliquots representing approximately fifty milligrams of bone ash were placed in the 4.75 square centimeter cups for activity measurements.

Total blood calcium was determined after the method of Clark and Collip (22). Total ash and phosphorus, and inorganic serum phosphorus were determined by the standard methods of procedure (35, 55). For clarity, the procedures used for each class of samples will be discussed separately:

Blood plasma: Citrated blood was centrifuged at 1500 revolutions per minute in an International Centrifuge (trunnion diameter fifteen inches) for thirty minutes and cell pack volume determined. The plasma was removed by suction and stored under refrigeration, usually for seven days before analysis. Total calcium was determined in duplicate or triplicate samples by pipetting two milliliters of plasma into fifteen milliliter centrifuge tubes containing one milliliter of saturated $(NH_4)_2 C_2O_4$. Two milliliters of distilled water was added, the solution mixed, allowed to stand for thirty minutes and then centrifuged. The precipitate was washed twice with five milliliters of ammonia wash reagent, dissolved in sulfuric acid and titrated with 0.02N $KMnO_4$. The radioassay was made by pipetting five to ten milliliters of plasma into a fifty milliliter conical bottom centrifuge tube to which was added an equal volume of a slightly acid solution containing eight milligrams of calcium carrier and three milliliters saturated $(NH_4)_2 C_2O_4$. The solution was thoroughly mixed, allowed to stand overnight, centriguged and the precipitate washed and collected in the plastic tube and metal cup assembly for counting. Briefly, the cup assembly (23) consisted of a tapered end polyethylene tube with a stainless steel cup forming the bottom. Tared cups were pressed securely over the end of the fitted tube until the assembly was watertight. The calcium oxalate precipitate was then washed quantitatively into the tube and the assembly placed in a fifty milliliter centrifuge carrier. To equalize the centrifugal pressure and to insure no leakage, water was placed between the tube and the carrier to a height equal to that in the plastic tube. Following centrifugation at 1500 revolutions per minute for twenty minutes, the supernatant was carefully siphoned off, the cups removed, and dried to a constant weight on trays under an infra red light. The samples were then placed in a dessicator for radioactivity measurements and weighed for total calcium and mass determinations.

The supernatant, containing the phosphorus was made to volume, and an aliquot estimated to contain approximately fifty micrograms of phosphorus was measured into another tube. One milliliter of fresh molybdate solution and then 0.2 milliliters of twenty five per cent one, two, four amino-napthol sulfonic acid were added to the aliquot. After standing thirty minutes for the color to develop, the solution was read in a Fisher photometer and compared with a standard containing ninety micrograms of phosphorus and a distilled water blank.

Soft tissues: At sacrifice, single tissue samples taken from rats, and approximately twenty grams of fresh tissue, taken in duplicate from cattle, were weighed into tared porcelain crucibles. The samples were dried overnight at 100° Centigrade and placed in a cold muffle furnace and the temperature raised stepwise over a period of twenty four hours to 600° Centigrade. When the ash was white, usually twenty four to forty eight hours later, the samples were removed and placed in a dessicator for weighing. If the ashing was incomplete, the crucible was cooled and the tissue treated with a few milliliters of perchloric acid with the usual precautions before re-ashing.

Following ash determinations, ten milliliters of 2N HCl was added, the ash transferred quantitatively to a volumetric flask or tube, made to volume with distilled water, and an aliquot representing fifty to 200 milligrams of ash transferred to a fifty milliliter conical bottom centrifuge tube. Three milliliters of saturated $(NH_4)_2\ C_2O_4$, were added to the solution in the tube, then one drop of 0.05 per cent methyl red and four milliliters of acetic acid (one to four) added slowly with mixing. The solution was adjusted to a pH of 5.0 by adding ammonium hydroxide (one to four) very slowly with constant stirring until just alkaline to methyl red, and then a few drops of acetic acid until the color was a faint pink. After standing overnight for complete precipitation of the calcium oxalate, the solution was centrifuged for twenty minutes at 1500 revolutions per minute and the supernatant gently removed for phosphorus determinations. The precipitate was washed with ten milliliters of NH_4OH (one plus forty nine) wash solution, recentrifuged and the supernatant added to the previous one. The precipitate was then treated with three milliliters of ten per cent H_2SO_4, heated at seventy degrees Centigrade for fifteen minutes to release the oxalate and titrated with dilute $KMnO_4$ according to standard procedure (115). Blanks and standards were run simultaneously for comparison.

The titrated sample was prepared for radioassay in the fifty milliliter centrifuge tube by adding one drop of saturated $(NH_4)_2 C_2O_4$, one drop of methyl red and neutralizing with NH_4OH (one plus four). Five milliliters of 2N HCl were added, then four of acetic acid (one to four), eight milligrams of carrier calcium and three milliliters of $(NH_4)_2 C_2O_4$ in that order. The pH was adjusted with NH_4OH and acetic acid and the solution allowed to stand overnight before centrifuging and transferring to the cup assembly previously described.

Bones and teeth: Single bone samples were taken from rats and duplicate or triplicate ten gram samples were carefully taken from cattle, weighed into tared porcelain crucibles and ashed in the muffle furnace at 650° Centigrade for twenty four to forty eight hours. The ashed bone was reweighed, ground to a powder in the crucible and after re-drying, 500 milligram samples were placed in a fifty milliliter volumetric flask, dissolved with 2N HCl and made to volume with distilled water. Aliquots of five milliliters were used for the precipitation of the calcium oxalate as previously described. The total calcium and the correction for self-absorption were calculated from the mass weight in the dry cup. Rat bones and cattle bone samples containing less than 500 milligrams of ash were dissolved in 2N HCL, made to volume with distilled water and an aliquot taken representing about fifty milligrams of bone ash.

Total phosphorus was determined as previously described on an aliquot of the original solution diluted to contain twenty to fifty micrograms of phosphorus.

Feces: Duplicate samples of approximately twenty grams of fresh feces from cattle and a twenty four hour collection from rats were dried and ashed as described for soft tissue. The ash was dissolved in 2N HCl and made to fifty milliliters with distilled water. The insoluble residue was permitted to settle and an aliquot representing about 100 milligrams of ash taken and treated as described for bones. The amount of calcium oxalate in the cup was sufficient for total calcium analysis and for radioassay. Phosphorus was determined as for bones and teeth.

Urine: Ten milliliters or a twenty four hour sample of urine from rats or duplicate twenty gram samples of fresh urine from cattle were ashed according to the procedure described for soft tissues and feces. The ash was dissolved in 2N HCl, heated to dryness, re-dissolved and made to a twenty five milliliter volume with 2N HCl and distilled water. A five milliliter aliquot was taken for total calcium analysis by titration with 0.02N $KMnO_4$ as described for soft tissue. Phosphorus analysis was made as previously described in detail. For radioassay, a ten milliliter aliquot was taken, eight milligrams of carrier calcium added, and precipitated for counting in the cup assembly.

PROCEDURE WITH CATTLE

Experimental

The cattle phase of this study involved forty-five selected Hereford calves, steers, heifers and cows ranging in age from a three month fetus to a 190 month old cow as described in Table 1. Except for the fetuses, these animals were maintained on calculated adequate rations previous to and during the experimental period. The young animals, including the month old calves, were fed fresh milk from a pail. The six month calves, were fed a ration of four pounds commercial calf chow meal and hay *ad libitum*. The older animals were selected from the farm herd and placed on experimental rations one to four months prior to using. This period of adjustment was necessary for conditioning to a constant ration intake and to the routine procedures. The C-2 ration consisted of four to six pounds of ground yellow corn, 0.5 pounds wheat bran fortified with 1500 International Units of Vitamin D in the form of irradiated yeast and a good grass or legume hay fed *ad libitum*. It provided a calcium and phosphorus intake well within the range for normal growth. Each animal was watered from a pail at regular intervals for adjustment to conditions prevalent during the experimental period in the metabolism units.

Following this conditioning period, the individual animals were placed in metabolism units as previously described (41) for the quantitative separate collection of urine and feces. Preliminary routine calcium and phosphorus balance trials were made, and after the

Table 1. Description of Experimental Animals and Treatments[1]

Age of Animals (months)	Animal Number[2]	Weight (pounds)	Ration	Method of Administration	Dose Administered Milligrams	Dose Administered Millicuries
(3)	F-1	1.1				
(3)	F-2	1.3				
(3½)	F-3	2.1				
6	F-4	14.1				
6½	F-5	18.8				
6	F-6	12.1				
8	F-7	45.0				
8½	F-8	70.0				
8½	F-9	75.0				
8	F-10	68.0				
8	F-11	49.0				
0.3	S-12	84.	Milk	Oral	11.1	0.3
0.3	S-13	72.	Milk	I.V.	11.1	0.3
0.3	S-14	78.	Milk	I.V.	27.0	0.5
1.0	S-15	120.	Milk	Oral	23.2	1.0
1.2	S-16	144.	Milk	Oral	73.9	0.5
1.5	S-17	175.	Milk	I.V.	16.4	1.0
6.0	S-18	342.	C-Meal[3]	Oral	90.0	1.8
5.0	S-19	350.	C-Meal	I.V.	161.0	4.7
6.0	S-20	388.	C-Meal	I.V.	161.0	4.7
6.0	S-21	458.	C-Meal	I.V.	161.0	4.6
7.0	S-22	436.	C-Meal	I.V.	161.0	4.6
15	S-23	481.	C-2	Oral	157.5	5.3
19	C-24	710.	C-2	Oral	3.8	2.9
20	C-25(3)[4]	614.	C-2	Oral	120.0	5.4
21	C-26(3)	690.	C-2	Oral	120.0	4.2
19	S-27	716.	C-2	I.V.	5.0	2.1
24	C-28(6)	936.	C-2	Oral	27.8	5.3
25	C-29(6)	873.	C-2	Oral	210.0	5.1
26	C-30(6)	736.	C-2	Oral	126.0	3.2
26	S-31	745.	C-2	Oral	68.7	2.6
29	C-32(8)	854.	C-2	Oral	165.4	5.0
26	C-33	1076.	C-2	I.V.	77.5	3.1

Table 1. (Continued) Description of Experimental Animals and Treatments[1]

Age of Animals (months)	Animal Number[2]	Weight (pounds)	Ration	Method of Administration	Dose Administered Milligrams	Millicuries
32	C-34(8)[4]	811.	C-2	Oral	157.5	6.0
30	C-35(3)	781.	C-2	Oral	135.5	3.9
36	S-36	1050.	C-2	Oral	129.2	4.3
36	S-37	1140.	C-2	I.V.	2.4	3.0
36	S-38	1157.	C-2	I.V.	1.3	1.0
34	C-39(8)	758.	C-2	I.V.	102.6	5.0
60	C-40	900.	C-2	Oral	165.3	6.0
73	C-41	740.	C-2	I.V.	145.0	4.2
144	C-42	980.	C-2	Oral	650.0	2.0
190	C-43	860.	C-2	Oral	90.0	2.0
144	C-44	997.	C-2	Oral	91.0	2.1
156	C-45	908.	C-2	I.V.	57.4	3.5

[1] All animals sacrificed seven days after oral or intravenous (I.V.) administration of calcium 45.

[2] Fetuses are designated by F, females by C and steers by S preceeding number.

[3] Purina Calf Chow meal.

[4] The number designates the month of gestation for the pregnant females.

animal had become adjusted to the unit, a single oral or intravenous dose of radiocalcium was administered (Table 1). Concurrent seven day chemical and radiocalcium balance studies were started.

Isotope administration: The procedures for administration by the oral and intravenous routes have been previously described in detail (45), but consisted essentially of administering to the immobilized animal calcium 45 as the chloride, adjusted to pH six in a minimum volume. For oral dosing, the activity was added to filter paper in a capsule of the appropriate size and administered with a balling gun. Intravenous dosing was performed by injecting the activity into a previously inserted polyethylene catheter and washing it quantitatively into the jugular vein with a small volume of heparinized normal saline solution (four milligrams heparin per 100 milliliters saline). A standard was prepared for each animal from an aliquot of the solution administered.

Blood, excreta, feed and tissue sampling: Blood samples were taken routinely during the seven day experimental period. For those animals dosed orally, an average of ten samples per animal were taken for analysis, using a regular bleeding needle. However, since it was desirable to study the disappearance rate following intravenous administration, an average of fifteen to twenty five periodic blood samples were taken from the jugular vein with a syringe through a prevously inserted polyethylene catheter (45). The bleeding catheter was left intact during the period of frequent sampling, usually three to twenty four hours and then removed. Lithium citrate was used in the collection flasks to prevent blood clotting. Hematocrit readings were recorded for each sample for calculation of total blood activity, and the plasma was removed by suction into a test tube for refrigeration until radioassay and chemical analysis were made.

Representative fecal and urine samples were taken in duplicate at regular intervals from the quantitative collections during the seven day period. Ration constituents and weighback were carefully ground and sampled for analysis and intake calculations. Since accurate water consumption records were not kept, the insignificant amounts of calcium and phosphorus intake from this source were not considered. All urine and fecal samples were refrigerated at thirty four degrees Centigrade until total calcium, phosphorus, ash and radiocalcium determinations were made.

Following the 168 hour balance study all animals, except the two five year old cows, were stunned by a blow on the head, and suspended head down for complete bleeding from the jugular veins and carotid arteries. The carcass was opened down the abdominal midline and the tissues and organs removed, with care being taken to avoid contamination with intestinal contents in animals that had received radiocalcium orally, and with blood in those animals dosed intravenously. Total weights of the organs were recorded and the contents of the various portions of the gastro-intestinal tract were removed, weighed separately and sampled. Duplicate twenty gram samples of soft tissue and ten gram samples of bone were placed in tared porcelain crucibles and weighed immediately, previous to placing in furnace or drying oven.

The procedures were arranged so as to eliminate any significant error due to loss of weight in the fresh samples. Consideration was given to the necessity for representative sampling of soft tissues and analogous bone sections for comparative purposes between animals. The twelfth rib, the sixth lumbar vertebra, angle of the mandible, the first and second incisors, first molars and samples from the shaft and epiphysis of the right metatarsus as indicated by the autoradiogram in Figure 1, were used in this study.

One metatarsal bone from each animal was carefully removed and prepared for autoradiograms by the method of Lotz et al. (80). The freshly excised bone was cleaned of excessive tissue and mounted on a soft wooden board one-fourth inch thick by means of Duco cement. When dry, thin sagittal sections were sawed from each bone with an adapted band saw. The sections were then exposed to Kodak double-coated No Screen X-ray film for necessary periods, dependent upon the dose and route of administration, and developed in the usual manner.

Careful consideration was given during all these procedures to precautions necessary in the handling of radioactivity (45). Protective clothing was worn by personnel and all excreta, carcass residue and contaminated wastes were placed in barrels for subsequent burial. Possible cross contamination with other isotopes was prevented by using good housekeeping procedures, equipment and slaughter instruments designated for use with radiocalcium, and by routine monitoring of the area and facilities.

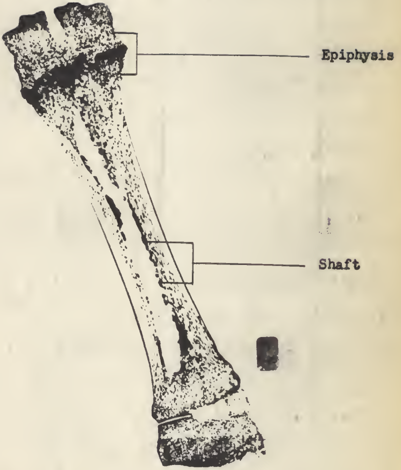

Figure 1. Autoradiogram of a bovine metatarsal bone indicating the areas from which samples were taken for assay.

Results and Discussion

Absorption and excretion: A summary of the individual calcium and phosphorus intake and excretion values for cattle of different ages is tabulated in Table 2. For convenience and comparative purposes average figures and the mean plus or minus the standard deviation (28) is presented for the groups at the various ages as indicated. Because of the difficulty of measurement of water consumption and the insignificance of the approximate 500 milligrams of calcium per day contributed, and since only traces of calcium were present in the urine, these values were not included in the chemical balance calculations. It is noted that in all groups the calcium and phosphorus intake level approached the required level as recommended by several investigators (91, 95) and that the intake per unit of body weight of both calcium and phosphorus decreased with increasing age. It is of particular interest that the retention of these elements decreased with increasing age in a similar manner. The per cent of calcium intake excreted was 7.2, 16.6, 76.0, 91.5, 88.3, 126.0, 109.0, and 136.0 per cent for animals 0.3, one, six, nineteen, twenty six, thirty four, sixty six, and 160 months of age respectively. The marked decrease in calcium and phosphorus absorption after one month indicates that the younger animals have the capacity for more efficient utilization either because of greater absorption or an increased tendency to readily incorporate the absorbed calcium into the body, or both. Positive calcium balance was largely maintained through the twenty six month old group, but in those animals over thirty months of age negative calcium and phosphorus balances

Table 2. Calcium and Phosphorus Balance Summary with Cattle at Various Ages

Age of Animals (months)	Wt. of Animals (lbs.)	Animal Number	Intake (gms/day) Ca	Intake (gms/day) P	Excretion (gms/day) Ca	Excretion (gms/day) P	Balance (gms/day) Ca	Balance (gms/day) P	Percent Intake Excreted Ca	Percent Intake Excreted P
0.3		S-12	9.7	10.8	0.47	1.2	+ 9.3	+ 9.7	4.8	11.1
0.3		S-13	8.2	9.1	.90	1.7	+ 7.3	+ 7.4	11.0	18.6
0.3		S-14	8.5	9.0	.51	1.5	+ 8.0	+ 7.5	6.0	16.6
Average	78		8.8	9.6	0.63	1.5	+ 8.2	+ 8.1	7.2	15.6
			± 0.88[1]	± 1.1	± 0.3	± 0.3				
1.0		S-15	7.3	6.3	1.1	2.4	+ 6.2	+ 3.9	15.0	38.0
1.2		S-16	6.9	6.0	1.8	2.8	+ 5.1	+ 3.2	26.0	46.6
1.5		S-17	8.6	7.3	0.83	1.4	+ 7.8	+ 5.9	9.6	19.1
Average	146		7.4	6.5	1.23	2.2	+ 6.2	+ 4.3	16.6	33.8
			± 1.0	± 0.8	± 0.6	± 0.8				
6		S-18	20.8	10.5	15.7	5.8	+ 5.1	+ 4.7	75.4	55.2
5		S-19	19.8	10.4	14.7	7.0	+ 5.1	+ 3.4	74.2	67.3
6		S-20	23.8	10.7	19.1	7.3	+ 4.7	+ 3.4	80.2	67.6
6		S-21	20.4	11.2	16.4	7.7	+ 4.0	+ 3.5	80.3	68.8
7		S-22	19.0	9.6	13.2	6.5	+ 5.8	+ 3.1	69.4	67.7
Average	493		20.8	10.5	15.8	6.9	+ 5.0	+ 3.6	76.0	65.7
			± 2.1	± 0.7	± 2.5	± 0.8				
15		S-23	23.7	10.4	20.6	10.2	+ 3.1	- 0.2	86.9	102.0
19		C-24	31.5	15.4	29.2	18.4	+ 2.3	- 3.0	92.6	119.0
20		C-25	17.6	14.0	15.5	16.0	+ 2.1	- 2.0	89.0	114.0
21		C-27	21.4	15.3	20.9	13.8	+ 0.5	+ 1.5	97.6	90.1
19		S-27	18.3	18.5	17.0	15.0	+ 1.3	+ 3.5	92.8	81.1
Average	642		22.5	14.7	20.6	14.7	+ 1.9	0	91.5	100.0
			± 6.0	± 3.5	± 5.9	± 3.5				

Table 2. (Continued) Calcium and Phosphorus Balance Summary with Cattle at Various Ages

Age of Animals (months)	Wt. of Animals (lbs.)	Animal Number	Intake (gms/day) CA	P	Excretion (gms/day) Ca	P	Balance (gms/day) Ca	P	Percent Intake Excreted Ca	P
24		C-28	17.7	19.9	15.5	17.3	+2.2	+2.6	87.5	86.9
25		C-29	18.2	18.4	16.5	16.7	+1.7	+1.7	90.6	90.7
26		C-30	42.0	13.6	34.8	13.8	+7.2	-0.2	80.9	101.0
26		S-31	16.2	12.0	13.3	10.9	+2.9	+1.1	82.0	90.8
29		S-32	32.0	14.9	28.5	9.6	+3.5	+5.3	89.0	64.4
26		C-33	18.4	20.0	13.7	15.7	-0.3	+4.3	102.0	78.5
Average	870		24.0	16.5	21.2	14.0	+2.8	+2.5	88.3	84.8
			± 10.2	± 3.2	± 8.6	± 3.1				
32		C-34	14.0	9.1	14.7	15.0	-0.7	-4.9	105.	164.
30		C-35	40.0	13.0	38.7	17.5	+1.3	-4.5	97.	135.
36		S-36	22.4	21.4	27.8	32.9	-5.4	-11.5	124.	154.
36		S-37	14.8	19.1	22.7	17.9	-7.9	+1.2	153.	94.
36		S-38	16.6	20.9	27.8	21.5	-8.8	-0.6	167.	102.
34		C-39	16.0	20.0	24.0	19.0	-8.0	+1.0	150.	95.
Average	950		20.5	17.1	25.9	20.6	-5.4	-3.5	126.	120.
			± 10.4	± 4.9	± 9.6	± 7.2				
60		C-40	17.5	14.6	20.2	14.0	-2.7	+0.6	115.	96.
73		C-41	20.0	17.0	21.0	18.2	-1.0	-1.2	105.	107.
Average	820		18.8	15.8	20.6	16.1	-1.8	-0.3	109.	102.
			± 2.2	± 2.1	± 0.7	± 3.4				

Table 2. (Continued) Calcium and Phosphorus Balance Summary with Cattle at Various Ages

Age of Animals (months)	Wt. of Animals (lbs.)	Animal Number	Intake (gms/day) Ca	Intake (gms/day) P	Excretion (gms/day) Ca	Excretion (gms/day) P	Balance (gms/day) Ca	Balance (gms/day) P	Percent Intake Excreted Ca	Percent Intake Excreted P
144		C-42	18.0	16.0	26.0	-	+ 8.0	-	144.	-
190		C-43	19.7	18.8	27.9	21.1	- 8.2	- 2.3	142.	112.
144		C-44	13.6	21.6	19.6	18.6	- 6.0	+ 3.0	144.	86.
156		C-45	20.2	18.2	23.2	-	- 5.0	-	115.	-
Average	936		17.9	18.5	24.2	20.0	- 6.4	- 1.5	136.	108.
			± 3.4	± 2.4	± 4.1	± 2.2				

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[1] Mean ± standard deviation.

were indicated. When the ration of twelve year old cows was supplemented with 100 grams of calcium phosphate in capsules on the first day of a seven day balance study, or when it was added at the rate of forty grams per day for five consecutive days of the balance period in four trials with two cows, the animals still remained in negative calcium balance. This would indicate that there was a decreased absorption in older animals, and that oral therapy of additional calcium, even when adequate phosphorus was present, did little to improve the negative balance prevailing.

Simultaneous radiocalcium balance studies with these animals substantiated the findings relative to decreased utilization efficiency with age as demonstrated by the total calcium findings. Table 3. illustrates the apparent effect of increasing age upon the fecal analysis, and upon the excretion of labeled calcium when administered orally and intravenously to cattle. For convenience of analysis and comparative presentation these thirty four animals were lotted into six age groups and the data with the mean plus or minus the standard deviation tabulated (28). The close agreement in fecal analytical values between groups is demonstrated. A higher concentration of ash, calcium and phosphorus was found in the feces of the younger animals. This may have been partly due to a dilution factor, since the quantity of feces excreted was small. It was also noted that the fecal calcium to phosphorus ratio was higher in the younger groups, decreasing with age from 2.2 in calves to 1.2 at 160 months.

Following oral administration of labeled calcium, it was observed

Table 3. A Summary of Fecal Analysis and Excretion of Labeled Calcium by Cattle of Various Ages

Age of Animal (months)	Number of Animals	Fecal Analysis (fresh weight)				Percent Ca 45 Dose Following Administration			
		Percent Ash	Mg. Ca (gm.)	Mg. P (gm.)	Ca/P	Oral		Intravenous	
						Feces	Urine	Feces	Urine
0.3	3	2.4 ± .59[1]	5.0 ± .36	1.85 ± .27	2.2	3.0	0.01	2.0 ± .13	0.07 ± .05
1.0	3	2.4 ± .60	6.70 ± 1.60	3.10 ± .44	2.2	6.5 ± 5.0	0.36 ± .60	0.94	0.01
6.0	6	2.2 ± .40	3.25 ± 1.04	1.84 ± .35	1.8	62.2 ± 2.0	0.07 ± .05	8.3 ± 1.5	0.06 ± .03
19-26	11	1.7 ± .58	1.90 ± .68	1.50 ± .22	1.3	66.0 ± 10.0	0.17 ± .12	13.8 ± 4.0	0.28 ± .46
30-60	8	1.6 ± .24	2.40 ± .42	1.70 ± .48	1.4	68.0 ± 12.1	0.18 ± .10	18.0 ± 3.2	0.43 ± .12
160	4	1.8 ± .49	2.70 ± .86	2.35 ± .35	1.7	82.5 ± 3.5	0.22 ± .11	25.0	0.35

[1] Mean ± standard deviation.

that the excretion in the urine was low, averaging for all ages less than 0.5 of one per cent of the dose. The fecal excretion ranged from three per cent in the young calf to 62.2 per cent in the six month old calves, and increased to 82.5 in the 160 month animals. It was of interest to note that the excretion of dietary calcium as measured by calcium analysis (Table 2.) was in every case higher than as measured by oral calcium 45 assay. Assuming that calcium 40 and calcium 45 are similarly utilized by the bovine, this has been interpreted to mean that a part of the calcium in the feces is derived from sources other than the feed, presumably from the body stores (42, 49). The details of this partition of fecal calcium as influenced by age will be treated more fully in a later discussion (Table 4.).

Figure 2. demonstrates graphically the accumulative fecal excretion of orally and intravenously administered calcium 45 by cattle of different ages during seven day balance studies. The kinetics of this process, as demonstrated by the rate and per cent of the dose excreted, were of particular interest. Following oral administration it was shown that total excretion by ten day and thirty day old animals was very low, and the rate was relatively constant. However, with animals six months of age the excretion rate was much higher than noted in the calves and the total per cent excreted was greater. The initial excretion rate of the six month animals was greater than that of the older animals, but this was reversed after seventy two hours and the older animals ultimately excreted more. The time lag evidenced in the excretions by the older animals represents a decreased rate of

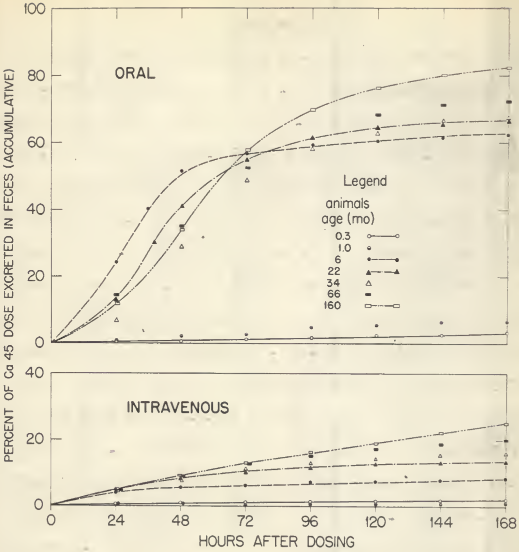

Figure 2. Accumulative fecal excretion of orally and intravenously administered calcium 45 by cattle of various ages during 168 hour balance periods.

gastro-intestinal movement, a decreased rate of absorption from the tract and a decrease in the rate of re-excretion of absorbed calcium. These retarded retes reflect the differences in the physiological state of the old and young animals.

The excretion curve for the intravenously dosed animals represents the secretion rate into the tract of that labeled calcium not fixed in the body. This again offers an overall picture of the physiological behavior of the animal at the different ages. The greatest excretion occurred during the first twenty four hours in all groups. Thereafter an apparent linear excretion was observed in all age groups, with the rate increasing with increasing age.

In Table 3. it is noted that the excretion values for the urine were generally higher following intravenous administration than after ingestion, but the total amount excreted was small in either instance. It was of interest that total excretion values in the younger animals differed little whether the isotope was administered orally or intravenously, but in the older animals dosed by the latter method there was a decrease in the rate of excretion, and an increase in total per cent excreted. This has been interpreted to mean that the calcium 45 is retained and incorporated into the tissues, especially the bones, of the younger animals faster than in those of the older animals. It is indicated that with increasing age, metabolic activity is retarded, total absorption by the bones is less, and the rate of calcium exchanged appears to be somewhat slower. The fecal excretion values following intravenous administration have been employed for the estimation of the endogenous fecal calcium (1, 54).

Blood calcium 45 concentration: Figure 3. illustrates the amount of labeled calcium in the total blood of cattle as a function of age and time following the administration of a single oral and/or intravenous dose. Total blood was calculated according to the blood volume findings of Hansard et al. (43), employing twelve, 8.5, 5.8 and 5.7 per cent of body weight for animals ten days, one, six and eighteen to 160 months of age, respectively. These data represent average figures for twenty animals in the oral group and fourteen in the intravenous group and the averages of approximately fifteen periodic blood sample values for each animal (Table 1.).

The rapid rate of removal of the labeled calcium following intravenous administration was striking. Ten minutes after injection, forty per cent of the dose was found in the blood of the mature and aged animals, twenty seven per cent in the six month old animals while only fifteen to eighteen per cent remained in the blood of the ten and thirty day old calves. At one hour these values had dropped to twenty, twelve and three per cent of the dose and at twenty four hours the values were four, one and 0.2 per cent respectively. The blood values up to one hour after injection are represented as an insert to show that this curve is not a straight line during this initial period. This difference in rate and quantity of labeled calcium released is in agreement with the excretion values in Table 3. and Figure 2. The lag in the curve of the older animals is probably due to the decreased bone uptake. The rapid disappearance cannot be considered as removal of excess calcium

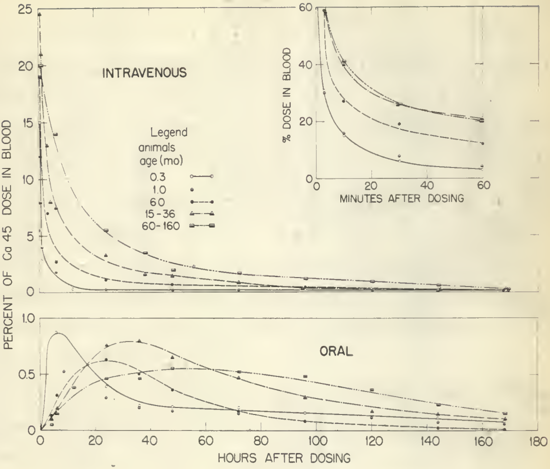

Figure 3. Calcium 45 in the total blood of cattle as a function of time and age of animal, following oral and intravenous administration.

from the blood however since there was no change in total calcium in the blood and this pattern was observed in studies where less than 2.0 milligrams of total calcium was administered (Table 1).

Thomas _et al._ (125) have made mathematical analysis of similar curves in young and old rabbits. They found that four exponential terms were needed to represent the data. From the first two terms they calculated the rates of exchange of calcium between plasma and the extravascular compartments and found it to be the same in both young and old animals. The third term in the blood disappearance equation was correlated with the bone uptake of calcium. The bone uptake by the young was greater than in the adult group. Therefore, this third period was postulated as a measure of bone activity. The last term from the equation expressing blood disappearance rates was correlated with the excretion of calcium from the body and was indicated to be an inverse function of the bone uptake of calcium. Armstrong _et al._ (3) presented evidence that calcium binding by plasma proteins does not impede the transcapillary movement of calcium, and that distribution of calcium between the ionized state and that bound to plasma proteins is a rapid process (67).

Following oral administration of labeled calcium to the bovine the calcium 45 ions are absorbed from the tract into the blood and presumably are removed at the same rate as indicated above. Figure 3. shows graphically the rate of appearance in the blood of calcium 45 orally administered to cattle of various ages. It is noted that the ten day calves had the highest level in the blood at six hours after administration.

Peak levels for the six month, mature and aged animals were at twenty four, thirty six and fifty hours, respectively. This would mean that the greater portion of dietary calcium was absorbed by these times after ingestion (50). These curves reflect two opposing processes - absorption from the tract and removal from the blood, and both are greater and faster for the younger animals.

Fecal calcium partition and its significance: A quantitative estimate of the partition of calcium in the gastro-intestinal tract of an animal would be of interest and of considerable importance as a basis for interpretation of the data procured from radioisotope tissue distribution studies. It has been demonstrated by concurrent chemical and radiocalcium balance trials with rats and with cattle (46, 51) that the per cent of a single oral dose of radiocalcium excreted is less than the per cent of the stable element simultaneously excreted in the feces. This has been interpreted to mean that the increased total calcium over the labeled calcium excreted represents the calcium from sources other than the feed (42, 47, 50). A measure of this endogenous calcium would be of value for the calculation of true digestibility, maintenance requirements and could be employed in the determination of net dietary calcium requirements for cattle at different ages; it may also serve as a measure of the current nutritional status of the animal (42, 47). Furthermore, since the principal pathway for calcium excretion is by way of the feces, a knowledge of the partition of this metabolic fecal calcium is important in the study of the overall calcium metabolism of the animal. Methods and techniques for the quantitative estimation of the excreted calcium and its partition within the gastro-intestinal tract of steers (42, 49, 51) and rats (42) have been previously presented and reviewed. It consisted essentially of integrating the excretion data of animals dosed orally with that from similar animals receiving a single intravenous dose of labeled calcium, and employing the seven day balance figures to calculate the source of the fecal calcium. For illustration the schematic diagram presented in Figure 4. shows the procedure and basis for the calculations, as a per cent of the ingested dose, of exogenous and endogenous fecal calcium, unabsorbed, absorbed, and the absorbed - re-excreted calcium in the feces of a six months old steer. It was assumed at the outset that tracer levels of calcium 45 chloride and the dietary calcium were equally absorbed from the tract and that the labeled calcium which reached the blood was secreted into the tract to the same extent as that of an intravenously administered dose.

Balance studies with these steers (Table 2.) indicated that seventy six per cent of the calcium intake was excreted in the feces. Concurrent radiocalcium balance figures (Table 3.) showed that only sixty two per cent of a single oral dose of the isotope was excreted. Results of the intravenous balance studies, however, indicated that in the six months old steer approximately eight per cent of the calcium 45 ions reaching the blood appeared in the feces. For illustration, it was assumed, therefore, that when a steer ingested (capsule) 100 units of calcium 45 then sixty two units of this would be excreted, and that which was absorbed (A) was calculated as follows:

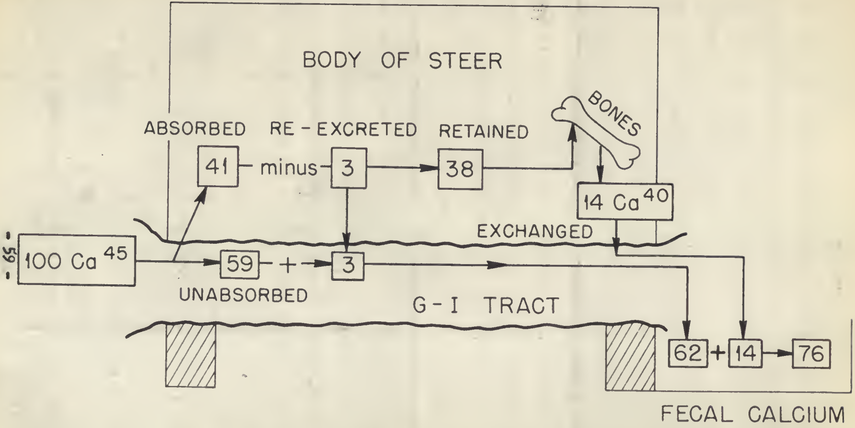

Figure 4. A schematic diagram of the partition of calcium in the gastrointestinal tract of a six months old bovine.

$$(100 - A) + (.08) A = 62$$

This means that forty one parts were absorbed into the blood of the animal. However, eight per cent of that calcium reaching the blood (forty one times .08) or three parts were re-excreted into the gastro-intestinal tract (Table 3.) leaving (forty one minus three) thirty eight parts retained in the animal body which were eventually deposited by accretion or exchange into the bones. It was calculated further that the total labeled fecal calcium excreted minus the calculated absorbed and re-excreted fraction (sixty two minus three) equaled the fraction of ingested calcium that was unabsorbed. The total calcium excreted then minus the unabsorbed labeled calcium (seventy six minus fifty nine) would equal to that portion of fecal calcium from sources other than the feed or the fecal endogenous calcium. Likewise, the calcium retained minus that exchanged (thirty eight minus fourteen) would indicate that twenty four per cent of the ingested calcium was deposited in the animal body.

It has been demonstrated earlier (46, 49, 51), however, that the difference between the total calcium and the excreted labeled calcium in the feces represented the fraction of total stable calcium exchanged for labeled calcium in the animal body (bones) under conditions of positive balance. This has been interpreted to indicate the current calcium status of the animal body (49). The results of this study are summarized in Table 4. and show the variations in the different fecal fractions with increased age and weight of the animal. In the first place it is observed that the unabsorbed calcium increased from

Table 4. The Partition of Calcium in the Gastrointestinal Tract of Cattle at Various Ages[1]

			Percent of calcium				Endogenous Fecal Calcium			Digestibility	
					Absorbed						
Age	Number of Animals	Average Weight pounds	Unab-sorbed	Ab-sorbed	Re-excre-ted	Re-tained	Total grams	Milli-grams kg[2]	Per cent[3]	"Apparent"	"True"
0.3	3	78	1.0	98.9	1.0	97.9	0.35	10.0	6.0	92.8	98.0
1.0	3	146	5.6	94.4	1.0	93.5	0.78	11.0	10.6	83.8	78.0
6.0	6	395	58.8	41.1	3.4	37.7	3.5	19.6	17.0	24.0	40.8
19-26	11	756	60.5	40.0	5.5	34.0	6.8	19.8	30.0	10.1	38.7
30-60	8	916	62.5	37.5	6.7	31.0	10.6	25.4	54.0	-15.0	40.5
160	4	936	76.7	23.3	5.8	17.5	10.6	24.8	59.0	-36.0	23.0

[1] Calculated from data in Tables 2 and 3.

[2] Milligrams per kilogram body weight per day.

[3] Expressed as percent of ingested calcium.

one in the ten day calf to nearly seventy seven per cent in the aged cow, and that the absorbed fraction decreased from ninety nine to forty one to twenty three per cent in the ten day, six and 160 month old animals, respectively. The calculated amount absorbed and subsequently re-excreted into the tract was two and six per cent for the young and the old animals, and the total calcium actually retained decreased rapidly from approximately ninety four in the thirty day old calf to thirty five in the mature animals and then to 17.5 per cent in old cattle. There appeared to be little difference in this retained fraction in animals from six to sixty months of age.

It was of interest to note that the per cent endogenous as well as the total grams of fecal endogenous calcium increased in a regular manner with age of the animal. When the endogenous calcium was calculated as a fraction of the total fecal calcium excreted the per cent decreased rapidly from 100 in the young calves to about thirty per cent in the six month old animal and remained relatively constant at this level. The total grams per day excreted increased from less than one in the young calf to three at six months and to ten grams per day in mature animals. The milligrams per kilogram body weight increased from ten in the ten day old calf to 19.6 in the six month old animals and to twenty five milligrams per kilogram per day in the feces of the aged cattle. These values indicate further, that although the proportional requirement for maintenance is small in the young growing animal (62, 110) it may amount to more than 500 milligrams per 100 pounds body weight per day for one month old calves.

These endogenous calcium values and those of the calcium balance (Table 2.) were used to calculate the "true" digestibility by the formula:

$$\frac{\text{Calcium intake} - \text{Fecal Calcium} + \text{Endogenous Fecal Calcium}}{\text{Calcium intake}} \times 100$$

and the figures compared to the "apparent" digestibility in Table 4. In the very young animals, it was noted that there was little difference between "apparent" digestibility as determined from the usual balance study and the calculated "true" digestibility. However, with increasing age the dependability of this comparison decreased, and at maturity the balance indicated the animals to be losing fifteen per cent more calcium from the body than ingested, when actually they were utilizing a third of the intake. This difference must then be contributed to the high percentage of fecal endogenous calcium in the older animals which amounted to more than fifty per cent in animals over three years of age.

Since the metabolic fecal calcium in ruminants makes up the major portion of the total calcium lost from the body, the maintenance requirements for cattle, which have not been previously directly estimated, would be essentially equal to the endogenous fecal calcium (56, 95). Requirements for growth, pregnancy and lactation have been estimated directly by product analysis, therefore, the summation of these should equal to the net calcium requirements. The use of these concurrent isotope and chemical balances permits a direct estimation of dietary utilization which may thus be used to calculate the dietary requirements from the net values.

Tissue ash and age: For the analytical data of this study, fifteen to thirty tissue samples were taken in duplicate from each of eleven fetuses and twenty nine cattle of various ages and analyzed separately. However, for brevity and clarity of presentation, only, the average values for blood and ten selected tissues are presented as the mean ± the standard deviation.

A summary of the percentage of ash in selected tissues of cattle at the ages indicated is shown in Table 5. There was no marked age effect in the soft tissue ash values. There was a slight upward trend, however, in per cent ash with increasing age in a few of the fetal tissues up to ten to thirty days after parturition, but for the most part after this time, the values were not significantly higher. The percentage and total ash was low in the fetal bones, increasing most rapidly during the second three months of the gestation period and then more slowly to maturity. Since these values were calculated from the fresh weights, consideration must be given to the higher moisture content of the tissues from the young animals. A comparison of the average ash values for the six bones reported showed a seventy per cent increase in ash to puberty, 100 per cent to ten days, 200 at eighteen months, and at 160 months the ash value was more than 300 per cent that of three month fetal bones. The spongy portions of bones having the higher metabolic activity showed the greater increase in ash per cent with increasing age. For example the epiphyseal sections of the metatarsus and rib, and the mandible at eighteen months showed a four to five fold increase in ash over corresponding samples from the three

Table 5. Percentage of Ash (fresh weight) in Selected Tissues of Cattle at Various Ages

Number of Animals	3	3	5	2	3	4	5	6	5	4
Age of Animals (months)	F-3.5	F-6.0	F-8.2	0.3	1.0	6.0	18.0	26.0	34.0	160.0
Blood Plasma	0.06 ± .01[1]	0.09 ± .03	0.07 ± .04	1.07 ± .05	1.02 ± .04	1.05 ± .09	0.90 ± .08	1.00 ±0.10	1.10 ± .12	0.90 ± .13
Gastrocnemius Muscle	0.84 ± .08	0.95 ± .03	1.00 ± .05	1.19 ± .12	1.15 ± .10	1.10 ± .05	1.13 ± .04	1.13 ± .03	1.08 ± .09	1.16 ± .07
Liver	1.38 ± .03	1.35 ± .11	1.40 ± .14	1.53 ± .21	1.42 ± .05	1.30 ± .06	1.39 ± .04	1.45 ± .06	1.40 ± .16	1.48 ± .07
Kidney	0.99 ± .10	1.0 ± .04	1.6 ± .06	1.31 ± .20	1.27 ± .11	1.31 ± .12	1.13 ± .12	1.14 ± .07	1.15 ± .15	1.14 ± .04
Spleen	1.23 ± .10	1.40 ± .12	1.4 ± .05	1.19 ± .39	1.38 ± .08	1.40 ± .10	1.36 ± .04	1.41 ± .06	1.31 ± .08	1.49 ± .12
Metatarsus Shaft	27.0 ±2.2	35.0 ±4.8	37.4 ±3.1	39.7 ±9.1	49.6 ±4.5	56.0 ±5.0	57.5 ±4.6	57.9 ±1.6	58.4 ±2.2	64.5 ± .2
Metatarsus Epiphysis	6.0 ±3.4	19.2 ±6.1	17.0 ±6.1	15.6 ±0.6	20.6 ±3.6	29.5 ±4.0	37.1 ±4.8	40.7 ±4.2	39.1 ±6.3	36.6 ±6.1
Rib Shaft	18.3 ±8.8	32.9 ± 4.4	30.8 ±3.4	37.0 ±5.2	38.0 ±1.7	37.0 ±3.5	46.1 ±2.9	46.7 ±4.8	46.7 ±5.0	48.1 ±5.3

Table 5. (Continued) Percentage of Ash (fresh weight) in Selected Tissues of Cattle at Various Ages

Number of Animals	3	3	5	2	3	4	5	6	5	4
Age of Animals (months)	F-3.5	F-6.0	F-8.2	0.3	1.0	6.0	18.0	26.0	34.0	160.0
Rib Epiphysis	7.5	19.3	17.6	20.6	17.2	18.0	21.2	22.2	22.0	33.1
	± 2.0	± 1.8	± 2.5	± 7.4	± 1.3	± 1.1	± 3.2	± 2.5	± 4.0	± 12.4
Mandible Angle	11.5	19.2	23.0	25.3	30.7	35.5	44.1	46.6	43.1	40.0
	± 5.3	± 3.1	± 4.5	± 2.0	± 2.1	± 2.0	± 1.7	± 7.2	± 1.6	± 4.4
Vertebra	5.7	8.8	12.6	20.3	18.2	25.0	26.9	33.7	36.4	43.5
	± .65	± 3.0	± 1.8	± 9.1	± .4	± 3.0	± 3.5	± 7.3	± 1.3	± .04

[1] Mean ± standard deviation.

month fetus, and then were relatively constant. In the aged cattle the rib and metatarsal shafts were increased slightly over that of the mature thirty four month old animals, but the epiphysis of these bones and the mandible were somewhat lower in the older groups. This would indicate that although these active bony tissues had ceased to grow they were still subject to resorption and thus function as a major storehouse for minerals even in old age. It is to be remembered, however, that ash percentage and total ash weight together are better criteria for assessing changes in the mineral status of bones from animals of variable ages or treatments than either alone (29, 94).

Tissue calcium and age: Table 6. shows the milligrams of calcium per gram for selected tissues of cattle from 3.0 month old fetus to 160 month old animal. It was noted that the soft tissue of the fetus, contained the highest concentration of calcium, with this value decreasing to parturition and then maintaining a relatively constant value. These data show no indications of significant increases in calcium content in the soft tissues of aged animals.

Calcium concentration in the selected bones increased rapidly during early gestation to six months and then less rapidly to eighteen months after which the calcium concentration paralleled the trends for the ash values previously described. A relative comparison of the calculated average value for the six bones reported indicated that from the third to sixth months of gestation calcium concentration doubled, increasing less rapidly then to six months of age when it was three times the value and at 160 months equaling four times that in the three

Table 6. Milligrams of Calcium per Gram (fresh weight) in Selected Tissues of Cattle at Various Ages

Number of Animals	3	3	5	2	3	4	5	6	5	4
Age of Animals (months)	F-3.5	F-6.0	F-8.2	0.3	1.0	6.0	18	26	34	160
Blood Plasma	.140[1] ± .047	.183 ± .020	.150 ± .039	.116 ± .027	.122 ± .041	.089 ± .019	.139 ± .029	.132 ± .016	.113 ± .025	.121 ± .002
Gastrocnemius Muscle	.161 ± .044	.103 ± .012	.061 ± .015	.046 ± .003	.038 ± .012	.050 ± .015	.046 ± .021	.056 ± .011	.047 ± .018	.058 ±.015
Liver	.110 ± .03	.080 ± .018	.062 ± .025	.074 ± .047	.057 ± .024	.078 ± .042	.061 ± .008	.083 ± .022	.075 ± .022	.092 ± .021
Kidney	.152 ± .04	.121 ± .012	.110 ± .029	.152 ± .05	.103 ± .02	.134 ± .062	.118 ± .091	.116 ± .027	.125 ± .03	.125 ± .027
Spleen	.141 ± .07	.102 ± .030	.066 ± .030	.146 ± .147	.064 ± .04	.077 ± .027	.066 ± .010	.089 ± .019	.072 ± .013	.077 ± .041
Metatarsus Shaft	92. ± 21.	138. ± 24.	138. ± 12.	165. ± 12.	189. ± 12.	213. ± 14.	225. ± 19.	208. ± 23.	217. ± 25.	250. ± 8.
Metatarsus Epiphysis	16. ± 5.	82. ± 39.	58. ± 18.	52. ± 11.	77. ± 14.	109. ± 18.	141. ± 20.	150. ± 16.	145. ± 10.	140. ± 20.
Rib Shaft	51. ± 7.	129. ± 16.	117. ± 14.	138. ± 22.	145. ± 4.	152. ± 13.	179. ± 11.	179. ± 21.	174. ± 17.	188. ± 25.

Table 6. (Continued) Milligrams of Calcium per Gram (fresh weight) in Selected Tissues of Cattle at Various Ages

Number of Animals	3	3	5	2	3	4	5	6	5	4
Age of Animals (months)	F-3.5	F-6.0	F-8.2	0.3	1.0	6.0	18	26	34	160
Rib Epiphysis	25.	69.	68.	69.	63.	81.	79.	77.	81.	127.
	± 4.	± 2.	± 10.	± 14.	± 5.	± 4.	± 13.	± 12.	± 16.	± 49.
Mandible Angle	35.	70.	80.	85.	116.	135.	171.	174.	164.	155.
	± 15.	± 14.	± 19.	± 5.	± 10.	± 12.	± 14.	± 16.	± 6.	± 20.
Vertebra	18.	31.	45.	65.	70.	88.	102.	128.	139.	168.
	± 3.	± 11.	± 8.	± 18.	± 4.	± 10.	± 14.	± 26.	± 4.	± 1.

[1] Mean ± standard deviation.

month fetal bones. The compact bone had a constant increase in concentration to eighteen months and leveled off, while the spongy bone continued to increase indicating that, after "maturity", it may still serve as a ready reservoir for calcium in the animal body.

Tissue phosphorus and age: The concentration of total phosphorus in selected tissues of thirty four cattle of various ages is summarized in Table 7. No values were presented for animals in the ten days and 160 month age groups. It was evidenced that the soft tissue phosphorus values more or less parallel those for calcium, except that a peak concentration was reached in the young fetus and was maintained thereafter relatively constant to thirty four months of age. The bones showed an expected increase in percentage phosphorus with age (54, 61). After about six months the compact bone reached a peak, but the concentration increased rapidly in the spongy bone to twenty six months. Comparative calculations of relative concentration from averages of the six bones reported indicated that phosphorus concentration increased more slowly than calcium. At one month after birth the phosphorus values doubled the three month fetal value and at thirty four months the concentration was three times this value. It is apparent therefore that phosphorus concentration in both bones and soft tissues follows closely the pattern noted for calcium.

Calcium to phosphorus ratios and age: In Table 8. the calcium to phosphorus ratios of selected cattle tissues have been calculated and summarized for comparison between ages. The mean plus or minus the standard deviation was not included in this table, since these were

Table 7. Milligrams of Phosphorus per Gram (fresh weight) in Selected Tissues of Cattle at Various Ages.

Number of Animals	3	3	5	3	4	5	6	5
Age of Animals (months)	F-0.3	F-6.0	F-8.2	1.0	6.0	18	26	34
Blood Plasma	.06 ± .01[1]	.05 ± .01	.07 ± .01	.06 ± .01	.07 ± .01	.06 ± .01	.06 ± .01	.06 ± .01
Gastrocnemius Muscle	1.67 ± .44	1.35 ± .55	1.70 ± .25	2.16 ± .30	1.94 ± .30	2.24 ± .80	2.15 ± .32	2.06 ± .28
Liver	2.45 ± .90	1.83 ± .98	2.60 ± .30	3.01 ± .40	2.70 ± .44	3.24 ±.39	3.20 ± .32	2.72 ± .48
Kidney	2.41 ± .48	1.74 ± .04	2.20 ± .22	2.58 ± .30	3.15 ± .35	2.57 ± .71	2.49 ± .68	2.43 ± .42
Spleen	3.31 ± .91	2.34 ± .69	2.9 ± .40	3.41 ± .41	3.05 ± .54	2.90 ± .53	2.65 ± .70	2.52 ± .55
Metatarsus Shaft	56. ± 7.	68. ± 18.	77. ± 21.	102. ± 18.	111. ± 28.	108. ± 19.	108. ± 10.	114. ± 7.
Metatarsus Epiphysis	9. ± 3.	41. ± 17.	31. ± 12.	43. ± 8.	55. ± 18.	68. ± 9.	78. ± 5.	77. ± 2.
Rib Shaft	41. ± 8.	69. ± 10.	58. ± 9.	71. ± 7.	80. ± 18.	87. ± 10.	87. ± 7.	88. ± 7.

Table 7. (Continued) Milligrams of Phosphorus per Gram (fresh weight) in Selected Tissues of Cattle at Various Ages.

Number of Animals	3	3	5	3	4	5	6	5
Age of Animals (months)	F-0.3	F-6.0	F-8.2	1.0	6.0	18	26	34
Rib Epiphysis	15.	30.	34.	35.	46.	42.	41.	43.
	± -	± 5.	± 4.	± 5.	± 10.	± 6.	± 5.	± 7.
Mandible Angle	24.	36.	42.	61.	49.[2]	85.	87.	86.
	± 5.	± 6.	± 9.	± 8.	-	± 8.	± 8.	± 2.
Vertebra	12.	17.	23.	37.	41.[2]	54.	64.	71.
	± 8.	± 5.	± 2.	± 4.	-	± 5.	± 11.	± 4.

[1] Mean ± standard deviation.

[2] Values available from only one animal.

Table 8. The Calcium to Phosphorus Ratio in Selected Tissues of Cattle At Various Ages.

Number of Animals	3	3	5	3	5	6	6	5
Age of Animals (months)	F-3.5	F-6.0	F-8.2	1.0	6.0	18	26	34
Blood Plasma (Inorganic)	2.33	2.20	2.14	2.05	1.85	2.32	2.28	1.82
Gastrocnemius Muscle	.01	.07	.04	.02	.03	.03	.03	.02
Liver	.04	.04	.02	.02	.03	.02	.03	.03
Kidney	.06	.07	.05	.04	.04	.05	.05	.05
Spleen	.04	.04	.02	.02	.03	.02	.03	.03
Metatarsus Shaft	1.64	2.01	1.88	1.85	1.92	2.08	1.92	1.90
Metatarsus Epiphysis	1.73	1.97	1.88	1.80	1.98	2.07	1.93	1.89
Rib Shaft	1.26	1.86	2.00	2.05	1.91	2.05	2.06	1.97
Rib Epiphysis	1.67	1.71	2.03	1.84	1.76	1.87	1.90	1.89
Mandible Angle	1.45	1.95	2.11	1.90	2.05	2.01	1.99	1.90
Vertebra	1.53	1.82	2.01	1.88	2.12	1.90	2.01	1.97

calculations from the average values of Table 5. In the soft tissues this ratio was relatively constant but showed more variation than that of bone. The ratios for the tissue of the young fetuses were low but increased with age and at 8.5 months were higher in most tissues than for the ten day old calves. Since the soft tissues develop first (117) during early fetal life there appeared to be an increased demand for phosphorus as shown by concentration ratios. The ratios for bone followed the same constant trend as that for the soft tissues, indicating that whenever or wherever calcium is deposited or resorbed a proportional fraction of phosphorus is involved. The slight decrease in this ratio after twenty six months may have been due to resorption taking place since many of these animals were in negative calcium balance (Table 2.).

Percentage of ash to milligrams of calcium per gram ratios calculated on the average bone values from these animals at different ages, excluding the three month fetal group, indicated a remarkably constant value of 2.64 ± .07. The ash to phosphorus ratios for the same six bones averaged 5.2 ± 0.15 for all ages. This substantiates earlier reports that calcium and phosphorus may well be calculated from the ash percentage values (46, 105) and further confirms the thesis (86, 87) that the mechanism by which mineral matter is lost from the skeleton is one of resorption and not demineralization.

Tissue distribution of labeled calcium: The deposition of labeled calcium in selected tissues of cattle of various ages sacrificed 168 hours after oral and intravenous administration of calcium 45 chloride is summarized in Table 9. Concentration values were expressed as per cent of the dose administered times ten quintuple per gram fresh weight and, for comparative purposes, were corrected to a body weight of 500 pounds. Excretion data previously presented (Table 3. and Figure 2.) indicated that retention of radiocalcium was greater following intravenous than for oral administration, especially in animals over thirty days of age. This was reflected in the concentration of calcium 45 in the tissues, since this ratio parallels closely the oral-intravenous retention ratio values. Method of administration appeared to have little effect upon tissue concentration in animals ten days or one month of age. However, at six months there was a marked difference in concentration in the bones; and in the eighteen month group and older, the values for both bone and soft tissue, following intravanous administration, ranged from two to six times that following oral dosing. This difference was more marked as the animal increased in age to thirty four months, and appeared less evident in the aged animal. This trend was further reflected in the older animals by the slower disappearance rate from the blood (Figure 3.). The higher calcium 45 concentration in the soft tissue of animals eighteen months of age and older may also be a reflection of the blood concentration values. Likewise, the lower values for the younger animals since they were sacrificed the same length of time after dosing, indicate equilibrium of circulating calcium 45 with that of the tissue, especially bones, to be more rapidly achieved than in older animals.

The concentration of labeled calcium in the bones was one to

Table 9. Concentration of Labeled Calcium[1] in Selected Tissues of Cattle of Various Ages at 168 Hours after Oral (0) and Intravenous (I.V.) Administration.

Number of Animals	1(0)1(I.V.)	2(0)1(I.V.)	1(0)4(I.V.)	4(0)2(I.V.)	5(0)1(I.V.)	3(0)2(I.V.)	1(0)1(I.V.)
Age of Animals (months)	0.3	1.0	6.0	18.	26.	34.	160.
Blood Plasma (0)	0.50	0.90 ± .35[2]	0.67	0.75 ± .46	1.04 ± .33	0.64 ± .30	1.57
Blood Plasma (I.V.)	0.45	0.36	0.67 ± .12	1.23 ± .13	1.57	2.51 ± .36	2.91
Gastrocnemius Muscle (0)	0.27	0.24 ± .04	0.19	0.42 ± .29	0.47 ± .12	0.36 ± .18	1.22
Gastrocnemius Muscle (I.V.)	0.20	0.21	0.38 ± .16	1.13 ± .16	1.20	0.94 ± .04	1.70
Liver (0)	0.26	0.25 ± .16	0.95	0.43 ± .17	0.48 ± .22	0.42 ± .07	1.50
Liver (I.V.)	0.21	0.27	0.52 ± .13	1.18 ± .34	1.56	0.82 ± .04	2.30
Kidney (0)	0.41	0.61 ± .29	0.44	0.96 ± .33	0.92 ± .14	0.65 ± .19	2.49
Kidney (I.V.)	0.34	0.59	0.87 ± .29	3.13 ± .12	3.36	2.13 ± .04	3.52
Spleen (0)	0.33	0.42 ± .30	0.23	0.51 ± .27	0.68 ± .15	0.52 ± .24	0.69
Spleen (I.V.)	0.24	0.43	0.50 ± .12	1.00 ± .30	2.68	1.29 ± .16	1.58

Table 9. (Continued) Concentration of Labeled Calcium[1] in Selected Tissues of Cattle of Various Ages at 168 Hours after Oral (0) and Intravenous (I.V.) Administration.

Number of Animals	1(0)1(I.V.)	2(0)1(I.V.)	1(0)4(I.V.)	4(0)2(I.V.)	5(0)1(I.V.)	3(0)2(I.V.)	1(0)1(I.V.)
Age of Animals (months)	0.3	1.0	6.0	18.	26.	34.	160.
Metatarsus Shaft (0)	257.	173.	60.	71.	79.	55.	76.
		± 27.		± 35.	± 28.	± 14.	
Metatarsus Shaft (I.V.)	213.	298.	202.	144.	246.	323.	188.
			± 72.	± 79.		± 45.	
Metatarsus Epiphysis (0)	282.	277.	78.	132.	92.	74.	129.
		± 112.		± 47	± 36	± 26	
Metatarsus Epiphysis (I.V.)	278.	330.	273.	259.	294.	499.	252.
			± 79.	± 73.		± 81.	
Rib Shaft (0)	689.	356.	272.	131.	119.	104.	91.
		± 39.		± 59.	± 38.	± 35.	
Rib Shaft (I.V.)	544.	435.	357.	400.	360.	415.	154.
			± 16.	± 51.		± 37.	
Rib Epiphysis (0)	543.	705.	395.	325.	362.	261.	87.
		± 62.		± 200.	± 108.	± 68.	
Rib Epiphysis (I.V.)	538.	680.	544.	802.	1180.	1000.	275.
			± 416.	± 298.		± 35.	
Mandible Angle (0)	403.	517.	233.	220.	247.	186.	108.
		± 4.		± 88.	± 150.	± 48.	
Mandible Angle (I.V.)	474.	606.	-	635.	790.	715.	475.
				± 160.		± 160.	

Table 9. (Continued) Concentration of Labeled Calcium[1] in Selected Tissues of Cattle at Various Ages at 168 Hours after Oral (O) and Intravenous (I.V.) Administration

Number of Animals	1(O)1(I.V.)	2(O)1(I.V.)	1(O)4(I.V.)	4(O)2(I.V.)	5(O)1(I.V.)	3(O)2(I.V.)	1(O)1(I.V.)
Age of Animals (months)	0.3	1.0	6.0	18.	26.	34.	160.
Vertebra (O)	396.	288. ± 42.	-	165. ± 85.	300. ± 129.	177. ± 9.	60.
Vertebra (I.V.)	492.	501.	-	518. ± 156.	895.	567. ± 263.	231.

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[1] Expressed as percentage of dose X 10^5 per gram fresh weight. (Calculations standardized to 500 pounds body weight).

[2] Mean ± standard deviation.

several hundred times that of the soft tissues. The higher concentration in the tissues of young animals following oral administration reflected the high per cent absorption and dose retention (Figure 2.) as well as the attainment of rapid equilibrium between the tissue and blood. However, the high concentration in the bones of the yearling and mature groups following intravenous administration indicated, as discussed later (Table 12.), either a retarded turnover rate or the increased ability of these tissues to retain the element tenaciously for use by the body. This marked tendency was not observed in the aged group, however, possibly because of a decreased blood supply to the bones (10) resulting in a decrease in the initial absorption of the labeled calcium. The more rapid accumulation in zones of active ossification than in compact bone can be explained as the outcome of the greater blood flow to these zones and the greater ease of penetration of the calcium ion through the organic matrix to the crystal surfaces. It was observed that calcium 45 uptake was more closely correlated to the structural type of the bone than to anatomical location.

The accumulation of orally administered calcium 45 decreased with increasing age of the animal. This was especially marked in the bone concentration values, and was less pronounced following intravenous injection. No doubt the major influencing factor involved was the differences in absorption and, with this marked variation, the effects of age were difficult to separate out and evaluate. A more feasible approach for interpretation, therefore, was to remove this variable by recalculating the values on the basis of "absorbed" instead of "administered" dose (42). This procedure minimized influences due to method of administration, excretion differences and individual animal variations, and permitted a comparison of the results as affected by the animal's physiological state. Table 10. presents data that more nearly represents variations due to animal physiological differences at the ages indicated, and substantiate the blood concentration curves previously discussed. The calcium 45 concentration in the soft tissues increased regularly from calfhood to maturity. There appeared to be five to ten times the concentration in the thirty four month animals as seen in the ten day old calf tissues; and in the aged cattle the values for blood, kidney and liver approached twenty to thirty times these values for young animals. It would appear then that age caused a definite increase in calcium accumulation in the soft tissues. However, this was not indicated by the total calcium analysis in Table 6. The high values must, therefore, be accredited to the decreased removal from the plasma by the bone in the aged animal and as a result more of the dose was available for distribution in soft tissue as indicated by the blood disappearance curves. Since the calcium of blood plasma is in equilibrium with that of the soft tissue (34, 120) this would indicate this equilibrium level to increase with increasing age of the animal.

The bone demonstrated less apparent changes due to age. The peak concentration at twenty six and thirty four months for such bones as the rib epiphysis, vertebra and mandible is difficult to explain, unless it was due to the fact that several of these animals were pregnant (Table 1.) and a renewed growth or increased activity of the Haversian

Table 10. Concentration of Absorbed Calcium 45 in Selected Tissues[1] of Cattle at Various Ages Dosed Orally (O) and Intravenously (I.V.)

Number of Animals	1(O)1(I.V.)	2(O)1(I.V.)	1(O)4(I.V.)	4(O)2(I.V.)	5(O)1(I.V.)	3(O)2(I.V.)	1(O)1(I.V.)
Age of Animals (months)	0.3	1.0	6.0	18.	26.	34.	160.
Blood Plasma (O)	0.51	0.96	1.77	2.20	3.06	2.00	8.96
Blood Plasma (I.V.)	0.47	0.35	0.73	1.43	1.82	3.05	3.87
Gastrocnemius Muscle (O)	0.28	0.26	0.50	1.23	1.38	1.13	6.97
Gastrocnemius Muscle (I.V.)	0.20	0.21	0.41	1.31	1.39	1.15	2.26
Liver (O)	0.26	0.27	2.51	1.26	1.41	1.31	8.57
Liver (I.V.)	0.21	0.27	0.57	1.37	1.81	1.04	3.06
Kidney (O)	0.41	0.65	1.16	2.82	2.70	2.03	9.22
Kidney (I.V.)	0.35	0.60	0.95	3.63	3.90	2.60	6.01
Spleen (O)	0.33	0.45	0.61	1.50	2.00	1.63	3.94
Spleen (I.V.)	0.25	0.44	0.55	1.16	3.10	1.51	2.10
Metatarsus Shaft (O)	264.	185.	160.	209.	232.	172.	234.
Metatarsus Shaft (I.V.)	213.	300.	220.	167.	285.	394.	250.
Metatarsus Epiphysis (O)	290.	296.	292.	388.	270.	233.	336.
Metatarsus Epiphysis (I.V.)	277.	333.	298.	300.	341.	609.	335.

Table 10. (Continued) Concentration of Absorbed Calcium 45 in Selected Tissues[1] of Cattle of Various Ages Dosed Orally (0) and Intravenously (I.V.)

Number of Animals	1(0)1(I.V.)	2(0)1(I.V.)	1(0)4(I.V.)	4(0)2(I.V.)	5(0)1(I.V.)	3(0)2(I.V.)	1(0)1(I.V.)
Age of Animals (months)	0.3	1.0	6.0	18.	26.	34.	160.
Rib Shaft (0)	710.	380.	719.	385.	349.	326.	520.
Rib Shaft (I.V.)	548.	439.	389.	464.	418.	506.	482.
Rib Epiphysis (0)	559.	754.	1044.	956.	1064.	817.	494.
Rib Epiphysis (I.V.)	527.	687.	593.	930.	1369.	1220.	366.
Mandible Angle (0)	403.	552.	616.	647.	726.	582.	617.
Mandible Angle (I.V.)	474.	612.	-	737.	916.	872.	632.
Vertebra (0)	395.	308.	-	485.	882.	554.	343.
Vertebra (I.V.)	511.	506.	-	600.	1038.	692.	307.

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[1] Expressed as percentage of absorbed dose X 10^5 per gram fresh weight (calculations standardized to 500 pounds body weight).

System stimulated circulation of blood to the bone resulting in greater absorption or exchange.

In order to determine if age affected the way calcium was distributed between soft tissue and bone, and between compact and spongy bone, the ratios of labeled calcium concentration between selected tissues are presented in Table 11. The kidney to muscle ratios demonstrated that the kidney concentration was about twice that of the gastrocnemius muscle, but increased age did not change this ratio materially. The concentration in the epiphysis as compared with the shaft of the metatarsus, was about 1.5, with no apparent age effect. The rib epiphysis to shaft ratios showed a definite peak at twenty six months that was difficult to explain, but which may have been due to the influence of pregnant heifers previously discussed.

The marked decrease with age in the ratios of the shafts of the metatarsus and the rib to the gastrocnemius muscle was of special interest. In the first place it indicated the magnitude of differences in calcium 45 concentration in the bone over that of soft tissues, and secondly that with increasing age there was a relatively low accumulation in bone as compared with muscle. The difference was especially marked when twelve year old animals were compared with the young calves. Seven days after administration of labeled calcium, the rib shaft of the young calf had 2500 times the concentration of the muscle, but in the 160 month animals this value was only seventy. Since this difference was not due to body weight or to differences in absorption, it means that the increased blood supply to the bones and the greater exchange

Table 11. Age and Distribution Ratios of Labeled Calcium[1] Concentration Between Selected Tissues in the Bovine

Age of Animal (months) Tissue and Ratios	0.3	1.0	6.0	18	26	34	160
Kidney Gastrocnemius Muscle	1.5	2.0	2.3	2.3	2.0	1.8	2.0
Metatarsus Epiphysis Metatarsus Shaft	1.1	1.6	1.2	1.9	1.2	1.4	1.7
Rib Epiphysis Rib Shaft	1.0	2.0	1.4	2.5	3.0	2.5	1.0
Metatarsus Shaft Gastrocnemius Muscle	940	710	320	170	170	150	60
Rib Shaft Gastrocnemius Muscle	2500	1460	1440	320	250	290	70

[1] Calculated as per cent of absorbed dose X 10^5 for 500 pounds body weight.

potential in the young animal during the period of rapid growth facilitated deposition and rapid equilibrium between the blood or muscle and the surface of the bone crystals. In a later comparison of specific activity ratios, further explanation of these differences with age will be treated more fully.

In order to visualize the effects of age upon the deposition pattern of orally administered calcium 45 in representative bones from these animals the autoradiograms of selected metatarsae are presented in Figure 5. The bones are, arranged from left to right, from ten day, five months, eighteen months and thirteen year old animals respectively. The dark areas represent calcium 45 deposition. For the ten day old animal it is obvious that the heaviest laydown was directly below the epiphyseal plate at the distal end, and that there was a well defined accumulation directly beneath the periosteum.

The "funnel effect" at the junction of the shaft and epiphysis is clearly shown. It is postulated that these dark funnel shaped lines in the subepiphyseal region indicate a means of growth of the epiphyseal area in a direction perpendicular to the vertical axis of the bone (24, 48, 76). The deposition at the bone ends represents both exchange and fixation of calcium by growth. This pattern is followed for the five month old animal and is less distinct in the eighteen month old steer. The discrete longitudinal streaks in the shaft probably represent the Haversian systems.

It is of interest to note that deposition beneath the plate is much less in older than in the younger animals, and reflects both the

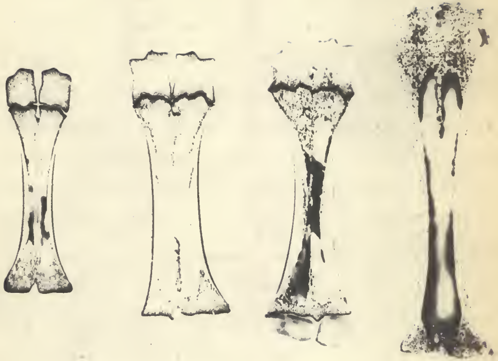

Figure 5. Autoradiograms of a sagital section from metatarsae bones of cattle. From left to right S-12, S-18, S-23 and C-42 represent ten days, six months, eighteen months and thirteen year old animals respectively.

decreased metabolic activity and the decrease in dose retention with age. In the aged animal this well defined deposition in the plate is missing, and there appears to be a more spotty accumulation in both compact and spongy bone with decreased localized deposition. These autoradiograms emphasize further the difficulty of procuring representative samples for radioassay.

Observations from a number of autoradiograms from these animals show that in the metatarsae of the bovine, growth is retarded at about nineteen months, as indicated by decreased metabolic activity in the area and the failure to show heavy deposition at the epiphyseal plate. Some demonstrated little growth at eighteen months, and only a few twenty four month old steers showed marked deposit beneath the plate. However, as indicated by the radiochemical concentration tables, the twenty six and thirty four month old group, composed largely of pregnant heifers, appeared to show some stimulation for increased laydown of calcium 45 in spongy bones. This was especially evident in the rib epiphysis as shown by the increased concentration and specific activity ratios over that of steers (Table 14.). Whether this is due to an estrogenic stimulation of growth, increased circulation through the Haversian canals, exchange, or to an increased ability to store readily available calcium for use by the fetus remains to be determined. The greatest effect appeared to be after six months gestation, the period immediately following the time when the concentration of fetal calcium is greatest (Table 5.), and when the greatest part of fetal calcium is being deposited (16). The absence of streaks in the autoradiograms of the aged animals may indicate that some or all of the Haversian systems have become inactive and blood supply to the bones thereby being greatly decreased, resulting in decreased deposition and exchange.

Tissue specific activity and age: A summary of the specific activities of selected tissues from cattle receiving a single dose of radiocalcium orally or intravenously at various ages is presented in Table 12. Data were expressed for comparative purposes as the per cent absorbed dose times ten quintupled per milligram of total calcium, corrected to a body weight of 500 pounds. When these values were compared on the basis of administered dose rather than per cent dose actually absorbed, it was observed that the specific activity (46, 51), following intravenous administration, was higher than after oral dosing, except in the very young animals where method of administration did not materially affect tissue calcium 45 concentration. Values for soft tissues were several times that for bones.

When these values were recalculated on the basis of absorbed dose, which has the advantages previously discussed, the comparison between tissues and between ages appears to be more revealing. The high ratio of the labeled calcium to total calcium between soft tissue and bone was reduced and the effects due to method of administration were for the most part eliminated. The soft tissues of the young calves demonstrated a lower specific activity with a regular increase to thirty four months, and then a marked increase in the 160 month old animals. These high values are in agreement with the blood disappearance curves previously presented, and indicate a slower removal by bone because of

Table 12. Specific Activity[1] of Absorbed Calcium 45 in Selected Tissues of Cattle at Various Ages Dosed Orally (0) and Intravenously (I.V.)[2]

Number of Animals Age of Animals (months)	1(0)1(I.V.) 0.3	2(0)1(I.V.) 1.0	1(0)4(I.V.) 6.0	4(0)2(I.V.) 18.	5(0)1(I.V.) 26.	3(0)2(I.V.) 34.	1(0)1(I.V.) 160.
Blood Plasma (0)	5.15	6.44	15.53	16.85	23.70	19.09	74.8
Blood Plasma (I.V.)	5.23	5.19	9.36	10.57	-	24.52	33.5
Gastrocnemius Muscle (0)	5.77	7.95	10.36	29.40	24.77	19.41	103.9
Gastrocnemius Muscle (I.V.)	4.49	4.24	8.28	26.22	23.20	25.50	45.2
Liver (0)	5.56	5.89	5.72	21.05	17.40	16.65	82.2
Liver (I.V.)	2.14	3.40	6.32	32.48	20.07	14.27	38.3
Kidney (0)	4.94	7.27	6.65	34.40	23.11	18.84	129.0
Kidney (I.V.)	3.26	6.60	7.47	17.40	35.38	17.32	43.0
Spleen (0)	5.36	7.67	8.40	23.05	21.96	22.28	73.1
Spleen (I.V.)	4.08	6.21	7.27	18.68	38.86	22.45	21.0
Metatarsus Shaft (0)	1.54	0.94	0.77	0.94	1.15	0.81	1.80
Metatarsus Shaft (I.V.)	1.38	1.68	1.01	0.82	1.33	1.73	1.00
Metatarsus Epiphysis (0)	4.94	3.50	2.20	2.76	1.82	1.66	2.84
Metatarsus Epiphysis (I.V.)	6.12	5.22	2.56	2.26	2.22	4.06	2.54

Table 12. (Continued) Specific Activity[1] of Absorbed Calcium 45 in Selected Tissues of Cattle at Various Ages Dosed Orally (0) and Intravenously (I.V.)[2]

Number of Animals	1(0)1(I.V.)	2(0)1(I.V.)	1(0)4(I.V.)	4(0)2(I.V.)	5(0)1(I.V.)	3(0)2(I.V.)	1(0)1(I.V.)
Age of Animals (months)	0.3	1.0	6.0	18.	26.	34.	160.
Rib Shaft (0)	4.74	2.56	5.22	2.10	1.88	1.88	2.90
Rib Shaft (I.V.)	4.39	3.11	2.54	2.83	2.82	2.94	1.00
Rib Epiphysis (0)	9.17	11.66	14.28	11.50	13.58	9.67	4.92
Rib Epiphysis (I.V.)	7.14	11.01	7.23	12.53	19.02	16.10	2.43
Mandible Angle (0)	4.74	4.77	4.74	3.70	4.17	3.54	4.30
Mandible Angle (I.V.)	5.92	5.27	-	4.85	5.32	5.38	3.82
Vertebra (0)	7.42	4.52	-	4.62	6.88	4.01	2.03
Vertebra (I.V.)	6.63	6.86	-	6.11	10.93	4.94	1.82

[1] Specific Activity - Expressed as per cent of absorbed dose X 10^5 per milligram of calcium (calculation standardized to body weight of 500 pounds).

[2] Animals sacrificed 168 hours after administration.

a decreased exchange rate or fixation as compared with the young animals. In the calf, equilibrium was apparently reached much more rapidly between the labeled calcium of the blood and that in the tissue than in the older animals, probably because of the higher absorption rate due to greater metabolic activity. The very slow loss indicates probable incorporation into the bone salts. There was no great difference between these ratios in soft tissue and bones in the young animals. Again this rapid attainment of equilibrium reflected in the blood curves indicated a more ready access of the blood plasma to the organic matrix of the bones of the younger animals.

The specific activity of bone showed an irregular increase with increased age to an apparent peak at twenty six to thirty four months of age, after which there was generally a decrease. This peak concentration of labeled calcium to stable calcium at these ages may have been an influence of the pregnant heifers included in these groups as previously noted under Table 10., and as later discussed in Table 14. The other values showed the expected trend as observed from the blood curves and as discussed earlier relative to calcium 45 concentration. The low specific activity in the bones of the aged animals may be explained on the basis of their decreased metabolic activity and decreased surface area exposed to the circulating blood, resulting in less uptake and less exchange taking place. The rib epiphysis, vertebra, mandible and metatarsus demonstrated the highest ratios and generally in that order for all ages.

Consideration must be given to the basic factors involved in calcium deposition and exchange for interpretation of these data. The labeled calcium is removed from the blood either by exchange or by accretion. *In vitro* studies have indicated (34, 119) that eighteen to thirty per cent of the calcium of bone may be involved in exchange. When calcium 45 exchanges into bone from the plasma it is replaced for the most part by stable calcium ions; at equilibrium, therefore, the concentration of calcium 45 in the bone will be several hundred times that in the blood. In the young growing animals the picture is further complicated by the greater proportion of the calcium ions leaving the blood being involved in bone accretion and the increasing rate of exchange, as indicated by the blood curves in Figure 3. The differences with age in the per cent of the calcium 45 dose actually absorbed, as calculated from the excretion curves in Figure 2., tends to mask a weighted interpretation of age effects unless corrections are made for these variables.

Table 13. is presented to isolate the effect of age upon the ratios of absorbed labeled calcium to total calcium between selected soft tissues, between soft tissues and bone, and between spongy and compact bone. These ratios appear to be a more reliable estimate of the order and rate of deposition, because they represent the amount of radiocalcium deposited as a function of the total calcium present in the tissue. It was noted that for kidney and muscle these ratios were about the same as for the calcium 45 concentration ratios and equal to approximately one. There was no age effect evident. Likewise, a comparison of the ratio of the metatarsus epiphysis to the shaft indicated the epiphysis

Table 13. Age and Specific Activity Ratios[1] Between Selected Tissues in the Bovine

Age of Animal (months) Tissue and Ratios	0.3	1.0	6.0	18	26	34	160
$\frac{\text{Kidney}}{\text{Gastrocnemius Muscle}}$	0.9	0.9	0.6	1.2	0.9	1.0	1.2
$\frac{\text{Metatarsus Epiphysis}}{\text{Metatarsus Shaft}}$	3.3	3.9	2.8	3.0	1.6	2.1	2.7
$\frac{\text{Rib Epiphysis}}{\text{Rib Shaft}}$	1.9	4.5	2.8	5.5	7.2	5.1	1.7
$\frac{\text{Metatarsus Shaft}}{\text{Gastrocnemius Muscle}}$	0.27	0.12	.07	.03	.05	.04	.002
$\frac{\text{Rib Shaft}}{\text{Gastrocnemius Muscle}}$	0.82	.32	.50	.07	.08	1.0	.03

[1] Calculated as per cent oral dose calcium 45 absorbed X 10^5 (corrected to 500 pounds body weight) divided by the milligrams total calcium per gram fresh weight.

to contain about three times the concentration of labeled calcium to stable calcium as the shaft. Again no marked age effect was noted, but there was a tendency toward a decrease in the older animals. However, the comparison of the rib epiphysis to shaft ratios showed the increase and peak at twenty six months previously discussed, followed by a marked decrease at 160 months. This further demonstrates the decreased metatolic activity of the spongy bone in the aged animal. The ratio of the shaft of the metatarsus and the rib to the gastrocnemius muscle illustrates the earlier demonstrated higher concentration of labeled calcium to stable calcium in soft tissue over bone. The decrease with age reflected previous observations, noted from the blood disappearance curves (Figure 3.) indicating that retarded equilibrium and slower turnover rates were characteristic of the physiological status of the aged animal.

In order to clarify the effects of pregnancy and period of gestation upon calcium behavior in the dam, and to estimate this probable influence upon the values here presented, the data in Table 14. were calculated. In this comparison, concentration in selected tissues of cows in the various stages of gestation were presented with those of steers of similar ages and nutritional background (Table 1.). It is noted that there was a slight increase in concentration in the bones of pregnant females over steers especially during the six months gestation period. These data are in agreement with the study by Plumlee *et al.* (105) where it was noted that the progression of mineralization in the fetal bones was at a maximum some time between four and seven months of gestation. This would indicate that the cow by some means

Table 14. Effects of Pregnancy Upon Calcium 45 Laydown and Relative Turnover Rates in Selected Tissues of Cattle.

Number of Animals Stage of Gestation	4 Steers	3 3 months	3 6 months	3 8 months
Calcium 45 Concentration[1]				
Metatarsus Shaft	180 ± 25	222 ± 108	237 ± 106	131 ± 10
Metatarsus Epiphysis	263 ± 40	417 ± 64	282 ± 4.7	172 ± 10.
Rib Shaft	280 ± 41	390 ± 145	369 ± 56	200 ± 15
Rib Epiphysis	731 ± 73	940 ± 627	1152 ± 309	733 ± 40
Concentration Ratios:[2]				
Metatarsus Epiphysis / Metatarsus Shaft	1.46	1.85	1.20	1.3
Rib Epiphysis / Rib Shaft	2.6	2.4	3.1	3.7
Specific Activity Ratios:[3]				
Metatarsus Epiphysis / Metatarsus Shaft	2.45	2.8	1.5	1.6
Rib Epiphysis / Rib Shaft	6.10	4.9	7.1	9.0

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[1] Calculated as per cent of absorbed dose X 10^5 for 500 pounds body weight.

[2] Calculated as the ratio of per cent dose of absorbed calcium 45 X 10^5 for 500 pounds body weight in tissues indicated.

[3] Calculated as per cent oral dose of calcium 45 absorbed X 10^5 (corrected to 500 pounds body weight) divided by the milligrams total calcium per gram fresh tissue.

must necessarily supply this increased calcium transfer, and in all probability would be correlated with a physiological change in her own tissues. A comparison of the concentration ratios of the rib and metatarsus epiphysis from these animals substantiates these suppositions, indicating a somewhat greater activity in these bones at these periods. The rib epiphysis was significantly higher (P = 05%) at the six months of gestation over that of the steers. The specific activity ratios, although less revealing for the shaft, indicated a definitely higher concentration of labeled calcium to stable calcium present in the rib epiphysis during the last quarter of the gestation period. These findings are in agreement with the earlier observations of Ellenberger _et al._ (31) who reported evidence that pregnancy increased the capacity for calcium retention.

It is shown by these data that the specific activity is influenced by many variables that may bias the calculated values. Therefore, when employed as a basis for the interpretation of results, limitations must be clearly defined and understood. When used for intercomparisons of relative tissue activities for one animal the application appears to be more appropriate than when indiscriminately used for the expression of differences due to treatments between animals.

PROCEDURE WITH RATS

Experimental

The rat phase of this study involved more than 200 selected animals from a highly inbred strain of Wistar rats (Table 15.). Littermate, weanling rats were marked, weighed and randomized into two groups. One group was placed on ration Ca-3, containing 0.4 per cent phosphorus and 0.5 per cent calcium; and the other group was placed on ration Ca-4, containing 0.4 per cent phosphorus and 1.0 per cent calcium. All animals were fed and watered *ad libitum*. They were maintained on these two rations, identical except for the difference in calcium content and cornstarch which was replaced by the calcium carbonate (Table 16.) for definite pre-experimental periods of time. At the ages indicated (Table 15.) the rats were removed at random from the two groups, given a single oral or intraperitoneal dose of labeled calcium (44) and placed in individual metabolism units for concurrent chemical and radiocalcium balance studies over a ninety six hour period.

Sampling of excreta, feed and tissue: Following isotope administration, total fecal and urine samples were collected separately (44), usually at twenty-four hour intervals, and the total periodic collection analyzed for calcium, phosphorus and radiocalcium by methods previously described in detail.

Feed was mixed weekly and an aliquot of each ration taken from each batch and composited for periodic chemical analysis. All animals were sacrificed by exsanguination after ether anasethetization, and the total blood collected in tared crucibles. Individual fresh tissue samples

Table 15. Description of Experimental Animals and Treatments[1]

Age of Animals (weeks)	Ration[2]	Number of Animals	Weight in grams[3] (average)	Ration Intake (grams)	Method of Administration[4]
1.5	Milk	6	14.0 ± 1[5]		Oral
4.0	Ca-3	29	37 ± 3	5.7 ± 0.99	Oral
	Ca-3	5	35 ± 4	5.8 ± 1.0	I.P.
	Ca-4	21	36 ± 4	6.2 ± 1.5	Oral
12	Ca-3	6	172 ± 17	8.9 ± 1.6	Oral
	Ca-3	7	180 ± 20	7.5 ± 0.9	I.P.
	Ca-4	6	212 ± 25	10.2 ± 1.2	Oral
	Ca-4	8	200 ± 30	9.7 ± 2.3	I.P.
24	Ca-3	28	300 ± 40	11.1 ± 1.3	Oral
	Ca-3	4	307 ± 49	10.9 ± 1.9	I.P.
	Ca-4	12	271 ± 43	11.4 ± 1.3	Oral
	Ca-4	4	304 ± 30	12.2 ± 2.0	I.P.
48	Ca-3	8	362 ± 66	11.5 ± 0.66	Oral
	Ca-3	4	360 ± 47	13.0 ± 5.[illegible]	I.P.
	Ca-4	8	350 ± 97	13.1 ± 3.2	Oral
	Ca-4	4	365 ± 58	14.2 ± 2.9	I.P.
72	Ca-3	9	520 ± 78	12.3 ± 2.3	Oral
	Ca-3	4	560 ± 16	12.8 ± 2.5	I.P.
	Ca-4	12	564 ± 145	14.2 ± 2.0	Oral
	Ca-4	4	535 ± 110	15.0 ± 3.0	I.P.
100	Ca-3	9	450 ± 87	15.0 ± 2.0	Oral
	Ca-4	6	482 ± 100	15.4 ± 3.2	Oral

[1] All animals sacrificed ninety six hours after isotope administration.

[2] For analysis, see Table 16.

[3] Average weight in grams at the time of dose administration.

[4] Animals received an average dose of twenty microcuries Ca 45 and 10-100 milligrams of calcium, except those animals under thirty days of age which received one half this amount.

[5] Mean ± standard deviation.

Table 16. Composition of Experimental Rat Rations[1]

Ingredient	Ration Ca-3	Ration Ca-4	UT-AEC Mineral Mixture[5] Ingredients	Per Cent
Corn Starch	642	630	$NaCl$	39.84
Casein (Vitamin-free)	180	180	KCl	18.20
Wesson Oil	40	40	$KHCO_3$	26.20
Vitab[2]	40	40	$MgSO_4$ (anhyd.)	12.00
Cellulose	40	40	$CuSO_4 \cdot 5H_2O$	0.40
Irradiated Yeast[3]	10	10	$MnSO_4 \cdot 4H_2O$	0.20
Cod Liver Oil[4]	2	2	$ZnSO_4 \cdot 7H_2O$	0.60
UT-AEC Mineral Mixture[5]	20	20	$FeCl \cdot 6H_2O$	2.50
K_2HPO_4	13.2	13.2	$K_2Al_2(SO_4) \cdot 2H_2O$	0.02
$CaCO_3$	12.3	25.0	KI	0.03
Per cent Calcium	0.5	1.0	NaF	0.01
Per cent Phosphorus	0.4	0.4		

[1] Expressed as grams per 1000 grams ration.

[2] Vitab purchased from Nopco Chemical Company, Harrison, New Jersey, and fortified with 0.4 milligrams riboflavin per gram.

[3] Irradiated yeast contained 360 I.U. Vitamin D per gram.

[4] Cod liver oil contained 90 I.U. Vitamin D and 900 I.U. Vitamin A per gram.

[5] See above mineral mixture for ingredients.

were taken from each rat immediately, in duplicate when possible, and placed in tared crucibles for routine calcium and phosphorus determinations and for measurement of calcium 45 (23). Consideration was given to the necessity of representative sampling from each animal for comparative purposes between animals and between age groups.

Results and Discussion

The initial design of this investigation, the maintenance of rats on rations of standard composition except for two levels of calcium intake, was based on the following considerations: (1) the utilization of dietary calcium decreases with age due either to decreased absorption or to poor assimilation. The feeding of an excess of calcium above the requirements for growth should help to differentiate between these factors. (2) There is a need for additional information on composition of animals that are brought to maturity and old age upon different dietary levels of calcium, other conditions being maintained the same. (3) There is a need for information concerning the effect of a normal and an excess dietary calcium level during the growth period upon the ultimate composition of bones during old age, and (4) the difference in response of paired groups of rats on different but constant levels of calcium nutrition may give more information on ageing effects than results from animals on a single dietary level.

Absorption and excretion: Table 17. shows a summary of fecal analyses from the rats maintained on the two levels of calcium intake until sacrifice at the ages indicated. These data represent values calculated from the ninety six hour balance studies with individual rats on the two rations, but for convenience of presentation only the average figures with the standard deviation (28) are presented. It is of interest to note that: (1) the weight of the daily feces increased with age and that those animals receiving the higher calcium intake (Ca-4) generally had the heavier feces. (2) The per cent ash was greater in the feces

Table 17. A Summary of Rat Fecal Analysis at Various Ages on Two Levels of Calcium Dietary Intake

Age of Animals (weeks)	Number of Animals	Ration	Average Dry Weight Feces (grams/day)	Percent Ash	Milligrams per gram Calcium	Milligrams per gram Phosphorus	Ratio Calcium/ Phosphorus	Ratio Calcium/[1] Ash
4	34	Ca-3	0.24 ± .06[2]	6.3 ± 1.9	21 ± 6.3	12 ± 4.3	1.8	3.3
4	21	Ca-4	0.23 ± .09	9.8 ± 2.1	31 ± 3.5	15 ± 3.0	2.1	3.2
12	13	Ca-3	0.68 ± .19	15.1 ± 1.5	37 ± 6.5	20 ± 5.1	1.9	2.5
12	12	Ca-4	0.95 ± .20	23.4 ± 1.7	75 ± 9.6	40 ± 2.3	1.9	3.2
24	28	Ca-3	0.82 ± .30	18.8 ± .76	54 ± 7.6	32 ± 6.6	1.7	2.9
24	12	Ca-4	1.07 ± .42	23.0 ± 2.0	85 ± 3.1	37 ± 7.0	2.3	3.7
48	8	Ca-3	0.78 ± .15	21.4 ± .15	70 ± 4.6	40 ± 2.6	1.8	3.3
48	8	Ca-4	1.08 ± .29	28.0 ± 1.3	109 ± 5.6	45 ± 4.6	2.4	3.9
72	9	Ca-3	0.97 ± .50	21.7 ± 1.4	65 ± 1.5	30 ± 2.0	1.9	3.0
72	12	Ca-4	1.48 ± .48	24.8 ± 2.8	98 ± 7.4	38 ± 2.9	2.6	4.0
100	9	Ca-3	1.36 ± .33	22.8 ± 1.6	67 ± 7.5	-	-	2.9
100	6	Ca-4	1.85 ± .90	24.0 ± 3.7	85 ± 6.9	-	-	3.6

[1] Calculated as milligrams calcium per gram (dry weight) divided by the per cent ash to give the calcium to ash ratios.

[2] Mean ± standard deviation.

of the older animals, but this difference was not affected by the calcium level. (3) The concentration of fecal calcium increased with age from twenty one milligrams in the four week old rats to seventy milligrams per gram in the feces of rats forty eight weeks old, and this increase was slightly greater in the high calcium groups indicating calcium absorption from the intestinal tract to be more dependent upon needs than the supply available. (4) Age or calcium intake level did not materially affect fecal phosphorus concentration after the animal was four weeks of age. However, the high dietary calcium apparently carried through some phosphorus as indicated by a slightly higher phosphorus level in the feces of this group. (5) The degree of differences in the calcium to phosphorus ratio of the two dietaries did not carry over into the feces.

Table 18. summarizes the calcium and phosphorus balance studies with rats of various ages on the two dietary levels of calcium. It is noted from these data that: (1) the dietary calcium level did not appreciably affect feed intake. (2) After three to six months of age, there was little change in total feed consumption, which indicated a marked decreased intake per unit of body weight with increased age. (3) The daily intake of calcium and phosphorus was of course a reflection of the feed ingested. (4) Total per cent of fecal calcium and phosphorus increased markedly up to six months and at a slightly reduced rate thereafter. A positive balance was maintained longer on the one per cent calcium intake level, but after twelve months, animals on both rations were in a negative balance at the time of the study. (5) The high calcium diet tended to cause an increased phosphorus excretion in

Table 18. Summary of Calcium and Phosphorus Balances with Rats of Various Ages on Two Levels of Dietary Calcium Intake.

Age of Animals (weeks)	Ration	Grams Ration Intake/Day	Daily Intake Milligrams		Daily Excretions Milligrams		Balance Milligrams		Percent Intake Excreted	
			Ca	P	Ca	P	Ca	P	Ca	P
4	Ca-3	5.7 ± 0.99[1]	28	23	4	3	+23	+20	14	13
4	Ca-4	5.8 ± 1.0	58	23	7	3	+51	+20	12	13
12	Ca-3	8.9 ± 1.6	45	36	25	14	+20	+22	56	39
12	Ca-4	10.2 ± 1.0	102	41	65	28	+37	+ 3	64	70
24	Ca-3	11.1 ± 1.3	56	44	44	26	+12	+18	79	59
24	Ca-4	12.4 ± 1.3	124	46	102	40	+12	+ 6	82	87
48	Ca-3	11.5 ± 0.66	57	46	55	32	+ 2	+14	96	70
48	Ca-4	13.1 ± 3.2	131	52	117	48	+14	+ 4	89	92
72	Ca-3	12.3 ± 2.3	61	49	63	34	- 2	+15	103	70
72	Ca-4	14.2 ± 2.0	142	57	145	56	- 3	+ 1	102	98
100	Ca-3	15.8 ± 2.0	79	63	91	-	-12	-	115	-
100	Ca-4	15.4 ± 3.2	154	52	157	-	- 3	-	102	-

[1] Mean ± standard deviation.

animals over one month of age.

Indications were that the older animals on both levels of dietary intake absorbed less calcium than the younger rats, either because their needs were less or their efficiency of utilization was decreased over that of the young animals. These problems are treated further in the next table.

The per cent of the current calcium intake utilized is indicated in Table 19. Which shows the accumulative per cent of administered dose of labeled calcium excreted by rats of various ages on the two levels of calcium intake following oral and intraperitoneal administration. These data indicate that: (1) excretion of orally administered calcium 45 was greater than when animals were dosed intraperitoneally. (2) Of the excreted labeled calcium, about ninety nine per cent was found in the feces and about one per cent was in the urine of rats of all ages. (3) Following intraperitoneal administration only a small fraction of the dose was found in the feces of animals under twelve weeks of age. However, after six months of age the quantity excreted in the feces increased to twenty five per cent of the administered dose and a significant amount was excreted by way of the urine. (4) There was less efficient utilization of the labeled dose on the higher levels of dietary calcium intake, regardless of method of administration, as shown by the higher levels of excretion on ration Ca-4 at all ages. However, there appeared to be less marked differences in animals over twenty four weeks of age.

(5) In consideration of the rate of excretion, it is interesting to note that: (a) in animals ten days of age excretion was near a

Table 19. The Accumulative Total Per Cent Administered Dose of Labeled Calcium Excreted By Rats of Various Ages on Two Levels of Calcium Intake Following Oral and Intraperitoneal (I.P.) Administration.

Age weeks	Ration	Method of Administration	Hours after administration 24	48	72	96 Feces	Total Excretion Urine
1.5	Nursing	Oral	0.40	.78	1.8	2.0 ± 5	0.29 ± .09
4	Ca-3	Oral	2.20	2.82	3.54	3.75 ± 1.0	0.50 ± .3
4		I.P.	0.25	0.45	0.65	0.73 ± 0.5	0.60 ± .2
4	Ca-4	Oral	3.10	4.5	5.1	5.6 ± 2.0	0.62 ± .5
4		I.P.	.35	.60	0.75	0.85 ± 0.6	0.50 ± .3
12	Ca-3	Oral	6.0	26.2	33.5	35 ± 14	0.27 ± .16
12		I.P.	2.1	2.8	3.1	3.2 ± 2	0.70 ± .66
12	Ca-4	Oral	10.2	35.5	49.7	53 ± 14	0.32 ± .14
12		I.P.	3.5	4.9	5.2	6.0 ± 3	0.98 ± .9
24	Ca-3	Oral	12.2	38.9	57.2	61 ± 7	0.30 ± .12
24	Ca-4	Oral	12.5	42.8	59.5	65 ± 13	0.36 ± .16
48-72	Ca-3	Oral	30.6	65.8	72.3	74 ± 13	0.50 ± .4
48-72		I.P.	5.7	10.5	20.0	21 ± 1	0.84 ± .13
48-72	Ca-4	Oral	38.5	68.0	74.1	75 ± 20	0.90 ± .50
48-72		I.P.	4.5	15.2	23.4	25 ± 1	1.0 ± .4

Table 19. (Continued) The Accumulative Total Per Cent Administered Dose of Labeled Calcium Excreted by Rats of Various Ages on Two Levels of Calcium Intake Following Oral and Intraperitoneal (I.P.) Administration.

Age		Method of		Hours after administration			Total Excretion
weeks	Ration	Administration	24	48	72	96 Feces	Urine
100	Ca-3	Oral	35.2	69.3	74.9	79 ± 13	0.33 ± .30
100		I.P.	3.9	14.1	21.6	25 ± 2	2.0 ± 1.0
100	Ca-4	Oral	40.2	72.3	81.7	83 ± 10	0.68 ± .65
100		I.P.	8.5	17.0	23.4	26 ± 3	3.0 ± 2.0

uniform level during the first seventy two hours, probably representing endogenous calcium. (b) At one month of age nearly fifty per cent of that excretion occurred during the first twenty four hours after oral administration, whereas at three months and six months the peak excretion following oral dosing was at forty eight hours; and, at twelve months and older an equal percentage excretion occurred at the twenty four and forty eight hour periods. This indicates that both speed of movement through the gastrointestinal tract and utilization was decreased with increasing age. (c) A somewhat similar pattern was noted following intraperitoneal administration. In the young animal, including rats three months of age, excretion was rapid, occurring principally during the first twenty four hours. With increased age, however, the peak excretion occurred during the second twenty four hour period after administration. At the end of ninety six hours the greater part of the dose to be excreted was found in the feces. The "spill over" into the urine probably reflects a lower threshold for calcium in the older animals. The time lag evidenced in the excretion of calcium in these animals represents the decreased rate of movement and mobilization, and reflects the physiological state of the aged animals as compared to the younger groups.

Partition of fecal calcium: Endogenous values for rats were calculated from Tables 18. and 19. and are presented in Table 20., as previously discussed for cattle. It is of interest to note from these data that: (1) the unabsorbed calcium was very low in four week old animals, was markedly increased at twelve weeks and twenty four weeks, and showed

Table 20. The Partition of Calcium in the Gastrointestinal Tract of Rats of Various Ages Maintained on Two Levels of Dietary Calcium Intake.

Age of Animals (weeks)	Ration[1]	Average Rat Weight (grams)	Per Cent of Calcium				Endogenous Fecal Calcim			Digestibility	
			Unabsorbed	Absorbed	Absorbed Re-excreted	Retained	Milligrams Per day	Milligrams kg[2]	Per Cent[3]	Apparent	True
4	Ca-3	37	3.	97.	0.7	96.	0.8	21	11.	86.	96.
	Ca-4	36	5.	95.	0.8	94.	0.5	14	7.	88.	95.
12	Ca-3	172	33.	67.	2.0	65.	6	35	23.	44.	58.
	Ca-4	212	50.	50.	3.0	47.	7	42	14.	46.	44.
24	Ca-3	300	56.	44.	5.0	39.	23	43	23.	21.	45.
	Ca-4	304	59.	41.	6.0	35.	28	90	23.	18.	40.
48-72	Ca-3	450	67.	33.	7.0	26.	26	47	33.	12.	37.
	Ca-4	460	67.	33.	8.0	25.	35	74	26.	12.	25.
100	Ca-3	450	72.	28.	7.0	21.	39	87	36.	-15.	20.
	Ca-4	482	76.	24.	6.0	18.	41	85	26.	- 2.	21.

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[1] For composition of ration see Table 16.

[2] Milligrams endogenous calcium per kilogram body weight per day.

[3] Expressed as per cent of ingested calcium.

only small further increases in animals at the older ages. In the younger animals this value was nearly twice as high with animals on one per cent calcium compared with those on 0.5 of one per cent calcium. After twenty four weeks, however, dietary intake had less effect on this value than did age of animal. (2) the per cent calcium absorbed, of course, was the reverse of this picture in that the younger rats absorbed ninety five per cent or more of the dietary calcium but at twelve weeks only about fifty per cent. At 100 weeks only twenty four to forty eight per cent was absorbed with little influence of dietary calcium intake level. (3) The calcium absorbed and subsequently re-excreted amounted to less than one per cent in four weeks old rats, but increased to three per cent at twelve weeks and leveled off at five to eight per cent in the older animals. (4) The net calcium retained decreased rapidly from ninety four per cent in the young rats to eighteen per cent in the aged animals. Dietary calcium intake level showed little effect, except that the rats on the higher calcium level retained, in every case, slightly less on a percentage basis than did those animals on a normal intake. (5) The endogenous fecal data showed an increase in per cent and total milligrams excreted with increased age. The fraction of the fecal calcium representing endogenous calcium showed no marked age effect, but was higher in the normal than the high dietary calcium rats. The data indicated that young rats have a maintenance requirement for calcium of about thirty five milligrams per kilogram body weight, or three times that of cattle. The total amount of endogenous calcium excreted per day and per kilogram body weight was greater on the Ca-4 ration at all ages. These data indicated that even during the periods of most rapid growth, fifteen to forty milligrams per day of calcium per kilogram body weight was required for maintenance. (6) True digestibility, as calculated from the endogenous calcium values and the balance data (Table 18.), decreased with increased dietary intake and with increased age but to a less extent than did the apparent digestibility. There was little difference between these values for four and twelve week old animals, but in older rats the efficiency of calcium utilization was much better expressed by the "true" digestibility figures. This is examplified by the 100 week old animals where the apparent digestibility indicated a negative balance, yet the true digestibility of calcium amounted to twenty per cent.

Tissue ash: For the analytical determinations, fifteen to twenty five tissue samples were taken from each of 180 rats maintained on rations Ca-3 and Ca-4 to sacrifice at the ages indicated. The samples were weighed, ashed and analyzed separately as previously described. However, for brevity and clarity of presentation, the average values for blood and nine selected tissues are tabulated as the mean ± the standard deviation.

A summary of the percentage of ash in the selected tissue of these two groups of rats sacrificed at seven ages is presented in Table 21. It was observed that: (1) the ash content of the soft tissues was not affected by either age or level of dietary calcium intake, except that the ten day old animals demonstrated a low ash percentage in the blood, muscle, kidney and spleen as compared to the older animals. (2) The bone ash showed no marked effects of dietary calcium intake level, but

Table 21. Percentage of Ash (Fresh Weight) in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)[1] and Ca-4 (1.0) to Sacrifice at Ages Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 14(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Blood Plasma	3	.71 ± .08[2]	0.96 ± 0.10	1.8 ± .14	0.92 ± 0.08	0.91 ± .02	0.86 ± .11	-
Blood Plasma	4		1.05 ± 0.44	1.03 ± .03	0.94 ± 0.07	.79 ± .01	0.82 ± 0.14	-
Gastrocnemius Muscle	3	.90 ± .06	1.32 ± 0.16	1.16 ± .01	1.18 ± 0.08	1.31 ± .33	1.04 ± .12	1.20 ± 0.10
Gastrocnemius Muscle	4		1.22 ± 0.09	1.23 ± .02	1.19 ± 0.03	1.08 ± .06	1.08 ± 0.08	0.15 ± 0.03
Kidney	3	1.09 ± .10	1.74 ± 0.57	1.62 ± .02	1.38 ± 0.13	1.40 ± .27	1.41 ± .20	1.20 ± 0.10
Kidney	4		1.45 ± 0.12	1.9 ± 0.17	1.19 ± 0.18	1.32 ± .29	1.30 ± 0.11	1.41 ± .20
Liver	3	1.44 ± .09	1.44 ± 0.77	1.21 ± .38	1.44 ± 0.03	1.40 ± 0.05	1.56 ± 0.26	1.52 ± .30
Liver	4		1.40 ± 0.11	1.28 ± 0.51	1.50 ± 0.12	1.30 ± .26	1.50 ± 0.28	1.41 ± 0.03
Spleen	3	1.33 ± .08	1.92 ± 0.51	1.68 ± .15	1.96 ± 0.35	-	1.75 ± .26	-
Spleen	4		2.1 ± 0.30	1.93 ± 0.12	1.62 ± 0.10	1.62 ± .10	2.26 ± .20	-

Table 21. (Continued) Percentage of Ash (Fresh Weight) in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)[1] and Ca-4 (1.0) to Sacrifice at Ages Indicated.

Number of Animals on Ration Age of Animals (weeks)	Ration	6(milk) 1.5	34(0.5) 21(1.0) 4	13(0.5) 14(1.0) 12	28(0.5) 12(1.0) 24	8(0.5) 8(1.0) 48	9(0.5) 12(1.0) 72	9(0.5) 6(1.0) 100
Tibia Shaft	3	5. ± 0.1	32. ± 3.6	47. ± 1.2	54. ± 1.2	54. ± 1.5	56. ± 1.1	40. ± 2.9
Tibia Shaft	4		28. ± 4.2	48. ± 2.0	52. ± 1.7	56. ± 1.7	56. ± 2.0	43. ± 1.3
Tibia Epiphysis	3	3. ± 0.9	23. ± 2.3	25. ± 1.8	38. ± 2.3	37. ± 1.0	38. ± 2.5	32. ± 4.2
Tibia Epiphysis	4		23. ± 7.1	28. ± 1.6	36. ± 3.0	34. ± 5.9	38. ± 6.9	31. ± 4.8
Incisors	3	26. ± 4.	62. ± 3.7	59. ± 1.4	68. ± 3.	68. ± 2.6	65. ± 1.80	67. ± 2.0
Incisors	4		54. ± 8.3	62. ± 3.0	68. ± 3.0	58. ± 9.4	63. ± 6.1	62. ± 3.7
Molars	3		50. ± 5.9	53. ± 6.8	61. ± 2.4	60. ± 3.0	59. ± 12.5	-
Molars	4		49. ± 8.5	54. ± 3.6	59. ± 3.0	61. ± 2.7	54. ± 7.7	-

Table 21. (Continued) Percentage of Ash (Fresh Weight) in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)[1] and Ca-4 (1.0) to Sacrifice at Ages Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 14(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Mandible Angle	3	15.	39.	47.	60.	40.	52.	43.
		± 3.	± 4.9	± 3.9	± 26.2	± 2.9	± 0.9	± 3.2
Mandible Angle	4		32.	47.	54.	51.	52.	37.
			± 11.4	± 3.8	± 1.36	± 1.5	± 2.4	± 5.2

[1] Refers to per cent dietary calcium intake (see Table 16.).

[2] Mean ± standard deviation.

did indicate a constant increase with age to seventy two weeks, and generally a slight decrease in the older group. The very low ash content of the bones of the ten day animals may have been due primarily to the higher moisture content, since ash was calculated from the fresh weight values.

Tissue calcium: The effects of age and dietary calcium intake level upon the concentration of calcium in selected tissues of rats are summarized in Table 22. The data in this table demonstrated that: (1) there was a wide variation in concentration between soft tissue samples from individual rats of the same age and on like rations. The standard deviation was as large for some tissues as the mean value itself, because of this wide range. (2) Soft tissues of the ten day old animals contained from two to three times the amount of calcium per gram fresh weight as did rats thirty days of age. This is indicative of the physiological functions of the rapidly growing animal and reflects the higher equilibrium level of plasma and new bones. (3) The level of dietary calcium intake did not appear to influence tissue calcium concentration significantly, indicating a threshold control for calcium either in the gastrointestinal tract or within one or more of the metabolic pools or both. (4) Soft tissues of animals after twelve weeks of age showed no indications of any marked increase in calcium concentration, except possibly the muscle and kidney. This increase in concentration was not significant, however, at the higher level of dietary calcium intake and would indicate a saturation limit (95) and relative rigid control by the physiological mechanisms regulating tissue calcium deposition even

Table 22. Milligrams of Calcium per Gram (Fresh Weight) in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)[1] and Ca-4 (1.0) to Sacrifice at Ages Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Blood Plasma	3[1]	0.120 ± .025[2]	.074 ± .037	.056 ± .048	.060 ± .002	-	.056 ± .029	-
Blood Plasma	4		.107 ± .047	.070 ± .03	.050 ± .002	-	.077 ± .041	-
Gastrocnemius Muscle	3	.298 ± .030	.078 ± .025	.074 ± .024	.090 ± .014	.106 ± .088	.089 ± .011	.111 ± .050
Gastrocnemius Muscle	4		.102 ± .046	.076 ± .025	.077 ± .048	.108 ± .018	.106 ± .024	.109 ± .060
Kidney	3	.208 ± .015	.097 ± .013	.135 ± .020	.124 ± .080	.156 ± .240	.144 ± .035	.141 ± .190
Kidney	4		.098 ± .047	.120 ± .060	.147 ± .025	.146 ± .052	.139 ± .020	.145 ± .029
Liver	3	.149 ± .031	.087 ± .040	.055 ± .014	.111 ± .024	.130 ± .020	.051 ± .033	.063 ± .040
Liver	4		.064 ± .022	.047 ± .017	.073 ± .041	.066 ± .017	.070 ± .020	.071 ± .028
Spleen	3	.291 ± .06	.078 ± .026	.069 ± .138	.080 ± .011	-	.070 ± .021	-
Spleen	4		.102 ± .016	.100 ± .081	.102 ± .068		.080 ± .042	

Table 22. (Continued) Milligrams of Calcium per Gram (Fresh Weight) in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)[1] and Ca-4 (1.0) to Sacrifice at Ages Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Tibia Shaft	3	85	115	159	162	170	202	215
		± 3	± 13	± 14	± 30	± 30	± 14	± 17
Tibia Shaft	4		104	168	160	204	212	219
			± 17	± 35	± 15	± 25	± 19	± 16
Tibia Epiphysis	3	70	83	95	119	144	156	157
		± 11	± 21	± 7	± 12	± 15	± 36	± 21
Tibia Epiphysis	4		72	111	119	139	154	158
			± 19	± 9	± 11	± 51	± 24	± 10
Incisors	3	114	207	209	248	242	241	211
		± 5	± 36	± 3	± 9	± 8	± 27	± 48
Incisors	4		189	206	225	227	234	255
			± 29	± 30	± 12	± 24	± 21	± 40
Molars	3		183	179	209	-	232	-
			± 12	± 12	± 33		± 60	
Molars	4		162	238	240	255	233	-
			± 42	± 78	± 37	± 55	± 41	

Table 22. (Continued) Milligrams of Calcium per Gram (Fresh Weight) in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)[1] and Ca-4 (1.0) to Sacrifice at Ages Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Mandible Angle	3	58 ± 4	135 ± 11	155 ± 32	161 ± 18	152 ± 17	202 ± 13	170 ± 18
Mandible Angle	4		135 ± 19	163 ± 18	165 ± 15	158 ± 23	166 ± 26	159 ± 43

[1] For ration analysis see Table 16.

[2] Mean ± standard deviation.

in the aged animals. (5) The calcium concentration in the bones followed the expected pattern (66), increasing steadily with age to forty eight or seventy two weeks, and then generally demonstrating a slight decrease. The low calcium concentration in the bones of ten day old animals could have been due to the high moisture content as compared with bones from the older animals, but is also indicative of the low level of calcification at which the rat starts life (122).

There were no consistent effects attributed to the higher dietary calcium intake level for any age; indicating further that once the tissues are saturated, the excess calcium is not absorbed (30, 95), or is excreted immediately after absorption. The parallel decrease, in the aged animal, of calcium concentration in the trabecular bones on both dietary calcium intake levels would suggest that the increased ingestion of dietary calcium alone for treatment during senescense may be of little value.

Tissue phosphorus: The concentration of total phosphorus in selected rat tissues as affected by age and dietary calcium intake level is summarized in Table 23. In consideration of the soft tissue values it is noted that: (1) the milligrams of phosphorus per gram were high in the blood and muscle of the ten day old rats, but values for the kidney, liver and spleen were lower than those of the four week old animals. (2) The tissue phosphorus concentration was not significantly affected by an increase in the dietary calcium intake level. (3) The tissue phosphorus concentration was apparently unaffected by age, except for an inclination to a slight decrease in the seventy two and 100 week old

Table 23. Milligrams of Phosphorus per Gram (Fresh Weight) in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)[1] and Ca-4 (1.0) to Sacrifice at Ages Indicated.

Number of Animals on Ration Age of Animals (weeks)	Ration	6(milk) 1.5	34(0.5) 21(1.0) 4	13(0.5) 12(1.0) 12	28(0.5) 12(1.0) 24	8(0.5) 8(1.0) 48	9(0.5) 12(1.0) 72	9(0.5) 6(1.0) 100
Blood Plasma	3	0.16 ± .09[2]	.05 ± .014	.075 ± .110	.080 ± .04	-	.057 ± .06	-
Blood Plasma	4		.073 ± .03	.068 ± .19	.069 ± .02	-	.08 ± .02	-
Gastrocnemius Muscle	3	3.95 ± .95	2.61 ±1.19	2.5 ± .95	2.18 ±1.05	2.33 ± .44	1.82 ± .22	2.0 ± .30
Gastrocnemius Muscle	4		2.26 ± .19	2.30 ± .27	2.63 ± .39	2.63 ± .21	1.88 ± .57	2.0 ± .41
Kidney	3	2.63 ± .88	3.23 ± .83	3.23 ± .61	3.33 ±2.26	3.91 ±1.06	2.91 ±1.41	2.8 ± .6
Kidney	4		2.99 ± .24	2.38 ±1.04	2.13 ±1.07	2.90 ± .60	2.29 ± .35	2.6 ±1.2
Liver	3	2.03 ± .20	2.48 ± .41	2.30 ± .59	2.30 ± .29	2.11 ± .15	2.01 ± .20	-
Liver	4		2.99 ± .27	2.60 ± .09	2.06 ± .31	2.16 ± .40	2.3 ± .52	-
Spleen	3	1.95 ± .80	3.51 ± .91	3.3 ±1.89	2.50 ± .19	-	2.64 ± .30	-
Spleen	4		3.69 ±1.04	3.00 ± .79	2.95 ± .28	-	2.26 ± .69	-

Table 23. (Continued) Milligrams of Phosphorus per Gram (Fresh Weight) in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)1 and Ca-4 (1.0) to Sacrifice at Ages Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (Weeks)	Ration	1.5	4	12	24	48	72	100
Tibia Shaft	3	38. ± 5.	62. ± 10.	81. ± 13.	92. ± 8.	94. ± 4.	97. ± 3.	92. ± 8.
Tibia Shaft	4		60. ± 8.	88. ± 19.	88. ± 25.	102. ± 35.	97. ± 8.	96. ± 4.
Tibia Epiphysis	3	36. ± 4.	47. ± 5.	53. ± 4.	66. ± 6.	74. ± 3.	75. ± 6.	68. ± 12.
Tibia Epiphysis	4		43. ± 13.	58. ± 2.	57. ± 6.	77. ± 8.	78. ± 14.	78. ± 10.
Incisors	3	47. ± 3.	112. ± 7.	119. ± 5.	127. ± 43.	145. ± 9.	146. ± 21.	130. ± 10.
Incisors	4		92. ± 16.	134. ± 11.	127. ± 12.	139. ± 14.	145. ± 5.	140. ± 16.
Molars	3		96. ± 13.	106. ± 7.	88. ± 16.	96. ± 6.	109. ± 21.	-
Molars	4		61. ± 17.	99. ± 6.	101. ± 5.	111. ± 7.	110. ± 12.	-

Table 23. (Continued) Milligrams of Phosphorus per Gram (Fresh Weight) in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)[1] and Ca-4 (1.0) to Sacrifice at Ages Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Mandible Angle	3	27.	73.	79.	77.	79.	89.	78.
		± 2.	± 6.0	± 13.	± 3.	± 6.	± 21.	± 10.
Mandible Angle	4		77.	83.	96.	87.	82.	88.
			± 11.8	± 5.	± 10.	± 2.	± 9.	± 13.

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[1] See Table 16. for ration analysis.

[2] Mean ± standard deviation.

animals. This was evidenced at both calcium intake levels, and may reflect the changing physiological status of the animal with increasing age.

In consideration of the concentration of phosphorus in bone it is indicated that: (1) phosphorus increases steadily with age, being low in the bones of animals ten days old and somewhat higher at four weeks, to a peak at forty seven to seventy two weeks and then a slight decrease in the 100 week old animals. (2) Dietary calcium levels fed appeared to have little influence upon phosphorus concentration in bone.

Calcium to phosphorus ratios: A summary of the calcium to phosphorus ratios of selected rat tissues as calculated from the phosphorus and calcium concentrations is presented in Table 24. These data indicate that: (1) there was no particular trend in this ratio with age in the soft tissue, nor was there a clear effect due to the dietary calcium intake level fed. (2) In the bone, however, there was less variation than noted in soft tissue ratios, and an apparent increase in the ratio in the tibia shaft and epiphysis and in the mandible of the seventy two and 100 week old rats. This pattern was the same on both levels of dietary calcium intake level, however, and the ratios were not significantly higher than that of the twenty four or forty eight week old animals. The calcium to ash ratios for the five bones analyzed averaged 3.5 for the rats on ration Ca-3 and 3.6 for those on the Ca-4 ration.

Tissue distribution of labeled calcium: The deposition after ninety six hours of orally administered labeled calcium in selected tissues of the rat at various ages, maintained on two dietary calcium levels, is summarized in Table 25. Concentration values were expressed

Table 24. The Calcium to Phosphorus Ratios in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)[1] and Ca-4 (1.0) Until Sacrifice at Ages Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Blood Plasma	Ca-3	0.74	1.48	0.75	0.75	-	0.93	-
Blood Plasma	Ca-4	-	1.46	1.03	0.72	-	0.96	-
Gastrocnemius	Ca-3	.075	.030	.030	.041	.046	.044	.056
Muscle	Ca-4	-	.044	.033	.030	.041	.059	.047
Kidney	Ca-3	.079	.030	.042	.037	.040	.051	.050
Kidney	Ca-4	-	.033	.050	.069	.050	.063	.056
Liver	Ca-3	.073	.035	.024	.048	.062	.025	-
Liver	Ca-4	-	.021	.018	.035	.032	.030	-
Spleen	Ca-3	.149	.022	.021	.032	-	.027	-
Spleen	Ca-4	-	.028	.033	.034	-	.035	-
Tibia Shaft	Ca-3	2.20	1.85	1.96	1.76	1.70	2.08	2.34
Tibia Shaft	Ca-4	-	1.73	1.89	1.90	2.00	2.18	2.28
Tibia Epiphysis	Ca-3	1.52	1.76	1.79	1.73	1.94	2.08	2.30
Tibia Epiphysis	Ca-4	-	1.70	1.91	2.09	1.80	1.97	2.02
Incisors	Ca-3	1.94	1.85	1.76	1.95	1.67	1.66	1.62
Incisors	Ca-4	-	2.05	1.54	1.77	1.63	1.61	1.82
Molars	Ca-3	-	1.91	1.69	2.37	-	2.13	-
Molars	Ca-4	-	2.66	2.40	2.38	2.30	2.12	-

Table 24. (Continued) The Calcium to Phosphorus Ratios in Selected Tissues of Rats Maintained on Rations Ca-3 (0.5)[1] and Ca-4 (1.0) Until Sacrifice at Ages Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Mandible Angle	Ca-3	2.15	1.85	1.96	2.09	1.92	2.27	2.38
Mandible Angle	Ca-4	-	1.75	1.96	1.83	1.82	2.02	1.81

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[1] Refers to per cent calcium in rations (see Table 16.).

Table 25. Concentration[1] of Calcium 45 in Selected Tissues[2] of Rats at Various Ages Maintained on Rations Ca-3 and Ca-4 to Sacrifice as Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Blood Plasma	3	.013 ± .002[3]	.015 ± .003	.015 ± .002	.007 ± .003	-	.014 ± .030	-
Blood Plasma	4			.009 ± .001	.009 ± .004	.013 ± .003	.011 ± .009	-
Gastrocnemius Muscle	3	.019 ± .005	.006 ± .004	.012 ± .002	.014 ± .005	.013 ± .001	.014 ± .004	.026 ± .013
Gastrocnemius Muscle	4		.014 ± .012	.007 ± .001	.009 ± .005	.006 ± .003	.005 ± .004	.031 ± .014
Kidney	3	.013 ± .01	.011 ± .005	.013 ± .01	.006 ± .001	.019 ± .004	.028 ± .008	.048 ± .023
Kidney	4		.002 ± .002	.011 ± .005	.007 ± .001	.007 ± .006	.008 ± .007	.040 ± .020
Liver	3	.042 ± .02	.004 ± .002	.005 ± .003	.006 ± .003	.010 ± .009	.022 ± .039	.022 ± .009
Liver	4		.002 ± .001	.003 ± .001	.004 ± .004	.004 ± .002	.004 ± .002	.024 ± .014
Spleen	3	.018 ± .003	.014 ± .005	.013 ± .002	.020 ± .001	-	-	-
Spleen	4		.003 ± .001	.012 ± .001	.005 ± .006	-	.008 ± .004	-

Table 25. (Continued) Concentration[1] of Calcium 45 in Selected Tissues[2] of Rats at Various Ages Maintained on Rations Ca-3 and Ca-4 to Sacrifice as Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Tibia Shaft	3	10.9	8.12	4.48	3.50	.714	1.16	0.80
		± 2.0	± 1.72	± 1.21	± 1.17	± .246	± 0.35	± .70
Tibia Shaft	4		7.10	3.03	2.20	1.16	.95	0.54
			± 3.21	± 0.26	± 0.49	± 0.33	± 0.45	± .28
Tibia Epiphysis	3	17.0	10.8	9.5	10.9	4.32	5.48	3.90
		± 3.5	± 1.33	± 4.1	± 5.3	± 1.54	± 3.06	± 1.30
Tibia Epiphysis	4		8.31	4.68	8.23	5.94	7.60	4.05
			± 2.46	± 1.4	± 8.4	± 3.1	± 3.90	± 1.74
Incisors	3	27.6	14.9	11.5	14.51	12.75	13.82	14.50
		± 4.2	± 3.72	± 2.5	± 1.44	± 1.81	± 3.31	± 3.98
Incisors	4		11.3	7.80	8.34	13.50	10.10	14.72
			± 1.88	± 1.35	± 1.13	± 3.65	± 4.71	± 2.15
Molars	3		8.94	4.78	5.22	4.08	2.89	-
			± 2.98	± 1.24	± 2.26	± 2.0	± 1.77	
Molars	4		6.82	3.37	3.63	3.46	2.30	
			± 1.13	± 1.2	± 1.03	± 1.78	± 2.1	

Table 25. (Continued) Concentration[1] of Calcium 45 in Selected Tissues[2] of Rats at Various Ages Maintained on Rations Ca-3 and Ca-4 to Sacrifice as Indicated.

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Mandible Angle	3	15.2	9.98	6.66	5.69	2.41	2.72	1.20
			± 4.16	± 3.20	± 1.25	± 0.39	± 0.49	± 0.52
Mandible Angle	4		8.53	5.58	5.51	2.91	2.00	1.46
			± 3.53	± 3.2	± 2.69	± 0.54	± 1.4	± 0.45

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[1] Expressed as percentage of dose labeled calcium per gram fresh weight (standardized to 100 grams body weight).

[2] All animals sacrificed ninety six hours after isotope administration.

[3] Mean ± standard deviation.

as percentage of the dose administered per gram of fresh tissue and calculations were standardized for comparative purposes to 100 grams body weight. These values for the rats dosed intraperitoneally with labeled calcium and sacrificed following ninety six hour balance studies were not included in this discussion because when concentration was calculated from the absorbed dose as discussed for cattle, their relationship to the values for the orally administered animals was comparable. In consideration of the tissue concentration data from those rats dosed orally, it is of interest to note that: (1) the concentration in the bones was several hundred times that in the soft tissue. (2) Concentration in the soft tissue at sacrifice tended to increase with age especially in the seventy two and 100 week old animals. (3) Concentration was generally higher in the soft tissues of those rats on the lower dietary calcium intake at all ages. (4) In the bones, there was a marked decrease with age in concentration of labeled calcium, except for the incisor teeth which remained relatively constant. (5) Calcium 45 concentration was generally lower in bones from animals that were maintained on the higher dietary calcium intake levels.

The excretion data (Table 19.) indicated a decreased retention of labeled calcium with increased age that was especially marked in animals older than twelve weeks. It was observed also that the differences between the dietary calcium groups was greatest at the younger ages. This difference was reflected in the tissue concentration data calculated from the administered dose, thus masking the effects due to age or to level of calcium ingested.

Therefore, in order to show more clearly the physiological differences caused by age itself, these values were recalculated on the basis of per cent absorbed calcium and the results summarized in Table 26. It is demonstrated by these data that: (1) there was a definite increase in soft tissue calcium 45 concentration with age, being most pronounced at seventy two and 100 weeks. However, since this was not shown by the total calcium values (Table 22.), the increase must be accredited to the decreased physiological functions particularly in the bones of the ageing animals, resulting in a slower removal of calcium from the blood, which being in equilibrium with soft tissues, resulted in higher calcium 45 values at the time of sacrifice. (Figure 3.) (2) Concentration of absorbed labeled calcium in bones showed less effect with increasing age, but demonstrated in general an increase to twenty four weeks of age and then a decrease. In contrast, the incisors demonstrated a constant and marked increase to 100 weeks on both normal and high dietary calcium intake levels. (3) Generally the bones from animals on the higher dietary calcium intake level showed the lower concentration of labeled calcium. This was to be expected from a simple stoichiometrical relationship. However, the fact cannot be overlooked that these bones may have been more highly saturated and calcium laydown with fixation was decreased, so that on the high calcium rations exchange was probably the principal mechanism for calcium 45 retention.

For comparison of the distribution pattern between different tissues in animals as a function of age and dietary calcium intake levels concentration ratios have been employed because they offer certain advantages.

Table 26. Concentration[1] of Absorbed Calcium 45 in Selected Tissues of Rats of Various Ages Maintained on Ration Ca-3 (0.5) and Ca-4 (1.0) to Sacrifice[2].

Number of Animals on Ration Age of Animals (weeks)	Ration	6(milk) 1.5	34(0.5) 21(1.0) 4	13(0.5) 12(1.0) 12	28(0.5) 12(1.0) 24	8(0.5) 8(1.0) 48	9(0.5) 12(1.0) 72	9(0.5) 6(1.0) 100
Blood Plasma	3	.013	.015	.023	.018	-	.052	-
Blood Plasma	4	-	-	.019	.026	.050	.044	-
Gastrocnemius Muscle	3	.02	.016	.018	.036	.052	.056	.124
Gastrocnemius Muscle	4	-	.012	.015	.026	.023	.020	.182
Kidney	3	.013	.011	.020	.015	.076	.104	.228
Kidney	4	-	.002	.023	.020	.027	.032	.235
Liver	3	.043	.007	.008	.015	.040	.088	.105
Liver	4	-	.002	.006	.011	.015	.016	.141
Spleen	3	.019	.014	.018	.051	-	-	-
Spleen	4	-	.003	.025	.014	-	.016	-
Tibia Shaft	3	11.2	8.36	6.85	8.96	2.86	4.29	3.81
Tibia Shaft	4	-	7.46	6.42	6.27	4.45	3.80	3.18
Tibia Epiphysis	3	17.5	11.10	14.54	27.90	17.28	20.28	18.56
Tibia Epiphysis	4	-	8.73	9.98	23.46	22.81	30.40	23.81
Incisors	3	28.4	15.30	17.60	37.12	51.00	65.69	69.02
Incisors	4	-	11.87	16.53	23.77	51.84	40.40	86.55
Molars	3	-	9.21	7.31	13.26	16.32	10.69	-
Molars	4	-	7.16	7.14	10.92	13.29	9.20	-

Table 26. (Continued) Concentration[1] of Absorbed Calcium 45 in Selected Tissues of Rats of Various Ages Maintained on Ration Ca-3(0.5) and Ca-4 (1.0) to Sacrifice[2].

Number of Animals on Ration		6(milk)	34(0.5) 21(1.0)	13(0.5) 12(1.0)	28(0.5) 12(1.0)	8(0.5) 8(1.0)	9(0.5) 12(1.0)	9(0.5) 6(1.0)
Age of Animals (weeks)	Ration	1.5	4	12	24	48	72	100
Mandible Angle	3	15.7	10.28	10.19	14.58	9.64	10.06	5.71
Mandible Angle	4	-	8.96	11.83	15.70	11.17	8.00	8.58

[1] Concentration expressed as percentage of absorbed dose calcium 45 per gram fresh weight (standardized to 100 grams body weight).

[2] All animals sacrificed ninety six hours after oral administration of calcium 45.

In a sense they permit the animal to serve as its own control. By using a standard tissue, such as the gastrocnemius muscle, for comparative purposes various uncertainties due to differences in absorption, bone accumulation, etc., are eliminated. It is felt, therefore, that this is the most satisfactory method for comparison between different age groups to demonstrate physiological differences due to age itself. In Table 27. calcium 45 concentration ratios in selected tissues of rats at various ages on the two levels of dietary calcium intake are demonstrated. The kidney to muscle ratios show a slight but significant increase with age in the labeled calcium concentration. However, this was apperently not affected by the added dietary calcium. Likewise the tibia epiphysis to shaft ratios demonstrate an increase with age, indicating a steady decreased metabolic activity in the shaft with age that was accentuated by the increasod dietary calcium intake level of ration Ca-4.

The tibia shaft to muscle and tibia epiphysis to muscle concentration ratios show the order of magnitude of differences in laydown of labeled calcium between bone and soft tissue. The decreased ratios indicated, that with increasing age, bone concentration was decreased markedly after forty eight weeks. This was probably due to both reduced exchange and contact of the bone crystals with blood calcium during the latter period of life.

Specific activity in selected tissues: The specific activity for the selected tissues of rats at various ages on the two levels of calcium intake was calculated as the per cent of absorbed dose of calcium 45 per milligram of calcium and corrected to 100 grams body weight. A summary of

Table 27. The Effects of Age and Dietary Calcium Level Upon Calcium 45 Concentration[1] Ratios Between Selected Rat Tissues.

Age of Animal (weeks) Tissue and Ratios		4	12	24	48	72	100
Kidney / Gastrocnemius Muscle	3[2]	0.7	0.9	0.4	1.5	1.8	1.8
Kidney / Gastrocnemius Muscle	4	1.0	1.5	0.8	1.2	1.6	1.3
Tibia Epiphysis / Tibia Shaft	3	1.3	2.1	3.1	5.7	4.7	4.8
Tibia Epiphysis / Tibia Shaft	4	1.2	1.6	3.7	5.0	8.0	7.4
Tibia Shaft / Gastrocnemius Muscle	3	525	380	250	89	76	31
Tibia Shaft / Gastrocnemius Muscle	4	621	428	241	193	190	17
Tibia Epiphysis / Gastrocnemius Muscle	3	690	800	770	332	360	149
Tibia Epiphysis / Gastrocnemius Muscle	4	727	665	902	991	998	131

[1] Calculated from the per cent absorbed calcium 45 dose in Table 25.

[2] Refers to rat ration Ca-3 and Ca-4 respectively.

these values is presented in Table 28. and indicates that: (1) the specific activity was higher in soft tissues than in bone except in the very young animals. (2) Specific activity increased in the soft tissue with increasing age, probably reflecting the decreased rate of absorption by bone and the decreased metabolic activity in the ageing animal (Figures 3. and 5.). (3) The attainment of equilibrium between calcium of bone and plasma, and the high percentage of exchangeable calcium in the bone of young animals was demonstrated by the low activity in the soft tissue and the relative high level in the bones of the ten day old animal. In the four week animals specific activity values were higher for some bones than for soft tissue, but at twelve weeks the increased turnover rate in the soft tissue over bone indicated a retarded equilibrium that progressed with age. (4) There was an apparent decreased specific activity in both bones and soft tissues of animals on the higher dietary calcium intake level as compared with animals on the normal ration, which is probably a simple mass effect as previously discussed.

In order to separate out and clarify the effects of age and of dietary calcium levels upon calcium behavior in the rat, the ratios of the specific activities of selected tissues are presented in Table 29. These data offer an estimate of the trend as well as rate and order of magnitude of laydown as a function of the total calcium present. The calculations were standardized to 100 grams body weight and corrected for absorption differences. The ratios of the specific activity of kidney to muscle showed that for animals on the Ca-3 ration, this ratio increased from 0.6 at four weeks to 1.5 in the 100 week old animals.

Table 28. Specific Activity[1] of Absorbed Calcium 45 in Selected Tissues of Rats at Various Ages Maintained On Ration Ca-3 and Ca-4 to Sacrifice[2]

Age of Animals (weeks) Tissue and Rations		1.5	4	12	24	48	72	100
Blood Plasma	3[3]	111.	209.	409.	300.	-	925.	-
Blood Plasma	4	-	-	271.	513.	-	572.	-
Gastrocnemius Muscle	3	66.	205.	248.	397.	488.	622.	1110.
Gastrocnemius Muscle	4	-	144.	195.	333.	215.	188.	1670.
Kidney	3	64.	116.	147.	123.	488.	718.	1620.
Kidney	4	-	51.	195.	137.	189.	232.	1620.
Liver	3	29.	47.	139.	138.	304.	1620.	1660.
Liver	4	-	33.	136.	157.	219.	228.	1980.
Spleen	3	64.	184.	288.	640.	-	-	-
Spleen	4	-	30.	254.	140.	-	400.	-
Tibia Shaft	3	132.	73.	43.	56.	17.	21.	18.
Tibia Shaft	4	-	71.	38.	40.	22.	18.	15.
Tibia Epiphysis	3	250.	134.	153.	246.	120.	130.	119.
Tibia Epiphysis	4	-	121.	89.	197.	165.	196.	153.
Incisors	3	249.	74.	84.	148.	212.	333.	328.
Incisors	4	-	63.	80.	105.	227.	172.	341.
Molars	3	-	50.	41.	64.	-	44.	-
Molars	4	-	44.	30.	46.	54.	40.	-
Mandible Angle	3	270.	76.	66.	90.	64.	48.	33.
Mandible Angle	4	-	66.	72.	94.	69.	48.	54.

[1] Specific Activity expressed as per cent of absorbed oral dose per milligram of calcium X 10^3 (calculation standardized to 100 gram body weight).

[2] Animals sacrificed ninety six hours after oral administration of calcium 45.

[3] Refers to rat ration Ca-3 and Ca-4 (See Table 16.).

Table 29. Specific Activity Ratios[1] of Selected Rat Tissues as Affected by Age and Dietary Calcium Intake.

Age of Animal (weeks) Tissue and Ratios		4	12	24	48	72	100
Kidney / Gastrocnemius Muscle	3[2]	0.6	0.6	0.3	1.0	1.2	1.5
Kidney / Gastrocnemius Muscle	4	0.4	0.9	0.4	0.9	1.2	1.0
Tibia Epiphysis / Tibia Shaft	3	1.8	3.5	4.4	7.1	6.2	6.6
Tibia Epiphysis / Tibia Shaft	4	1.7	2.3	4.9	7.5	10.8	10.2
Tibia Shaft / Gastrocnemius Muscle	3	0.4	0.2	0.1	0.03	0.03	0.02
Tibia Shaft / Gastrocnemius Muscle	4	0.5	0.2	0.1	0.1	0.09	0.01
Tibia Epiphysis / Gastrocnemius Muscle	3	0.7	0.6	0.6	0.2	0.2	0.1
Tibia Epiphysis / Gastrocnemius Muscle	4	0.8	0.5	0.6	0.8	0.9	0.1

[1] Calculated from the specific activity values in Table 27.

[2] Refers to rations Ca-3 and Ca-4 respectively.

This indicated a tendency for calcium to accumulate in the kidney with increased age, especially noted in animals over twenty four weeks old. However, this could be due to the comparative decrease in metabolic activity of the kidney with age as compared with the muscle; a greater turnover in muscle than kidney of aged animals, or the previously reported deposition in soft tissue of old animals (84). This same pattern was observed in animals on ration Ca-4.

The tibia epiphysis to shaft ratios likewise demonstrated a steady decrease in the metabolic activity of the cortical bone with increasing age. Rats on the Ca-4 ration showed a similar response, but more marked at seventy two and 100 weeks than did those animals on the lower dietary intake levels. The tibia shaft and epiphysis to muscle ratios substantiates these findings and indicates the changing physiological state to be altered in both shaft and epiphysis, but more drastically affecting the metabolic activity of the shaft cortical bone, than the trabecular bone of the epiphysis. The levels of dietary calcium intake did not appear to have a significant influence on these trends, but the greater sparing effect was on the epiphysis.

Estimation of skeletal mass: A simple isotope dilution method for the estimation of total bone mass in the animal body would be of particular interest to the physiologist and nutritionist. A method that would avoid the tedious process of determining individual bone weights after dissection and cleaning would make this procedure applicable for general use in the assessment of overall changes in the animal skeleton during growth, ageing, and in the course of experimental investigations. The absorbed calcium concentration in selected bones provides information from which a calculation of total skeletal weight may be made. The limitations, however, are that calcium 45 concentrations may vary widely between bones or parts of bones, and uniform representative samples are difficult to procure. It may be considered, however, that the skeleton is made up primarily of cortical bone and trabecular bone; for instance, the metatarsus or tibia shaft and epiphysis respectively. Therefore, uniform samples from these two areas may be used as representative of these two types of bone in the animal body, and the average calcium 45 concentration should represent the limits within which the concentration for the skeleton as a whole would fall. The ratio of cortical to trabecular bone in the body has not been determined, but this value for the aged cow has been approximated as sixty per cent cortical bone to forty per cent trabecular bone (13). However, tissue concentration data, autoradiograms and blood disappearance curves previously discussed for radiocalcium would indicate this ratio to be wider for young than old animals. The forty to sixty ratio for trabecular and cortical bone might be used in this estimation of total bone in the body without introducing a marked bias if calcium 45 concentration values are established after equilibrium is reached. If a relative shorter experimental period is used, however, data from in vitro studies indicate that equilibrium may not be complete (26), and the differences in concentration ratio between the two bone areas in young and old animals would affect the values more significantly.

It has been determined that about ninety nine per cent of the calcium in the body is in the skeleton (12, 31, 115, 117) and therefore with the above assumptions, the absorbed calcium 45 concentration values for the metatarsus or tibia shaft and epiphysis, as representative of cortical and trabecular bone respectively, have been used to estimate the total bone mass in the body of the rat as follows:

Let: B_C = the per cent absorbed dose calcium 45 per standard body weight, per gram of cortical bone.

B_T = the per cent absorbed dose calcium 45 per gram of trabecular bone (per standard body weight).

C = grams of cortical bone in body.

T = grams of trabecular bone in body.

X = fraction of total bone that is cortical.

1-X = fraction of total bone that is trabecular.

G = grams of total bone for standard body weight.

Then we know that:

(1) $C + T = G$

(2) $\frac{X}{1-X} = \frac{C}{T}$

(3) $(B_C)(C) + (B_T)(T) = 99$ (per cent calcium (16))

(4) $(B_C) C + (B_T)(C)\left(\frac{1-X}{X}\right) = 99$

(5) $C\left(B_C + B_T \frac{1-X}{X}\right) = 99$

Then:

(1) $C = \dfrac{99}{\left(B_C + B_T \frac{1-X}{X}\right)}$

(2) $T = \frac{C(1-X)}{X}$

(3) $G = T + C$

(4) $\frac{G}{\text{Standard body weight}}$ = per cent skeleton

(5) $\frac{(G)\ (\text{Body weight})}{(\text{Standard body weight})}$ = total skeletal weight of animal

The effect of age and dietary calcium intake level upon the total skeletal mass in rats is summarized in Table 30., as calculated from the above procedure. It is of interest to note that the estimated weight of the skeleton in relation to the total body weight decreased from values of ten to twelve per cent at thirty days of age to an average of about eight per cent at six months and remained relatively constant at that figure throughout the growth period to old age. Animals receiving one per cent dietary calcium had no heavier skeletons than those rats maintained on one half this calcium level. Total grams followed a similar trend with age but indicated a slight decrease in skeletal weight during the senescent period. These data agree rather closely with the values reported by Shchl (117) for the skeletal mass of the human body and with Mitchell's estimations (95) for the rat, but conform less closely with calculated skeletal mass values from Ellenberger's data for the bovine (31).

The difficulty involved in a practical procedure for the procurement of representative samples of cortical and trabecular bone, especially in cattle, and the task of determining the change in ratio with age of these bones in the animal body limits this procedure, even in rats where whole bones may be analyzed, to gross estimations of skeletal mass in the animal body.

Table 30. The Effects of Age and Dietary Calcium Intake Level Upon Estimated Total Skeletal Mass in Rats.

Age (weeks)	Weights	Ration	Concentration Absorbed Ca-45		C[1]	T[2]	G[3]	Total Bone	
			Tibia Shaft	Tibia Epiphysis				Grams	Per cent
4	37	3	8.4	11.1	6.3	4.2	10.5	3.9	11.
	36	4	7.5	8.7	7.4	4.9	12.3	4.4	12.
12	172	3	6.9	14.5	6.0	4.0	10.0	17.6	10.
	212	4	6.4	10.0	7.6	5.1	12.7	26.9	12.
24	300	3	9.0	27.9	3.6	2.4	6.0	18.0	6.
	271	4	6.3	23.5	4.5	3.0	7.5	20.3	8.
48	262	3	2.9	17.3	6.8	4.6	11.4	31.3	11.
	350	4	4.5	22.8	5.0	3.4	8.4	29.4	8.
72	520	3	4.3	20.3	5.5	3.7	9.3	48.4	9.
	564	4	3.8	30.4	4.1	2.7	6.8	38.4	7.
100	450	3	3.8	18.6	6.0	4.0	10.0	45.0	10.
	482	4	3.1	23.8	5.2	3.5	8.7	41.9	9.

[1] C = grams of cortical bone in body of rat calculated on basis of 100 grams body weight.

[2] T = grams of trabecular bone in body of rat calculated on basis of 100 grams body weight.

[3] G = (C+T), or grams of total bone in body of a 100 gram rat.

GENERAL DISCUSSION

Calcium metabolism in the bovine and the rat has been investigated using chemical and radioisotope techniques to study the fundamental problems concerned with the behavior of this element in the animal body, particularly as influenced by age.

The interpretation of these studies requires, however, that consideration first be given to the basic concepts of radiocalcium behavior in the normal animal. When calcium 45 ions are introduced into the blood either by intravenous injection or by absorption from the gastrointestinal tract, they are removed by the several competing processes, such as mixing with body fluids, deposition in the soft tissues and bones, or by excretion (13, 23, 26, 34, 48, 50). All of these processes take place rapidly as indicated by the curves for the disappearance of radiocalcium from the blood (Figure 3.), the feces excretion curves (Figure 3.), and by the data on equilibration of calcium 45 with the calcium in the blood, soft tissue and bone (3, 25, 34, 50, 105). The specific activities of the soft tissues and the blood rapidly approach an equal value, indicating that the calcium ions of the blood and those of the tissues are readily exchangeable (34). Likewise, the calcium 45 ions are incorporated into the bone by the process of exchange with calcium ions located on the surface of the bone crystal (60), by growth and possibly by the slower process of recrystallization (2, 101). The rate and extent of bone accumulation therefore will depend upon the relative kinetics of these processes as influenced by the amount of growth (26), exchange capacity, vascularity and resulting contact of the plasma with bone crystals (105,120).

The high total calcium concentration of bone as compared with that of plasma results in practically all of the calcium 45 in the blood eventually being deposited in the bone (51, 119), which, however, does not represent accretion of bone *per se* (34, 53).

The relative rates and magnitudes of these movements of calcium 45 can be used to give information on the behavior of stable calcium ions in the body (46, 47, 53), and are especially useful in the study of the effects of age upon calcium metabolism.

The results of concurrent chemical and radiocalcium balance studies with animals of different ages illustrate the application of these concepts and indicate that both calcium absorption and efficiency of utilization are decreased with advancing age. Old animals not only absorbed less calcium from the gastrointestinal tract, but re-excreted three to five times more of that which was absorbed than did the young animals (Tables 4 and 20). Since calculations were made from net excretion values, it is impossible to determine the differences due to "recycling" of the re-excreted calcium. However, it would appear that this was a contributing factor in calcium absorption in young animals. These findings also strengthen the evidence of earlier investigations (48, 58, 40) that in addition to poor absorption, there is decreased retention with age by the skeletal tissues themselves. This behavior was independent of dietary calcium intake level. It is obvious, therefore, that utilization is governed by internal mechanisms, but how these mechanisms control the absorption from the intestines or regulate the deposition and subsequent fixation of the absorbed calcium into the skeletal tissues is difficult to determine, and requires further study. The possibility that renal function is involved is ruled out because of the relatively small proportion of the calcium excreted by this route.

Endogenous fecal calcium values indicated negligible losses of total calcium from the body of young growing animals, but in yearling cattle, for example, this loss amounted to six grams per day, increasing sharply to more than ten grams per day in the mature and aged animals. The values for rats maintained on normal dietary calcium levels followed a similar pattern but at a higher level when expressed in milligrams per kilogram body weight. An excess of dietary calcium in the ration of rats from weaning to sacrifice appeared to have a slight sparing effect upon the total per cent of endogenous calcium, but did not reduce the amount excreted in this form. The endogenous loss by old cattle was not altered when excessive amounts of calcium were fed in a single dose or throughout the balance period.

The true digestibility figures indicated that utilization efficiency decreased rapidly during the first few months of life, but that after the age of sexual maturity in cattle and rats there was no further appreciable decrease until the approach of senescense. During the latter period, however, both absorption and utilization efficiency were again decreased, and this increased loss from the body was not alleviated in cattle or rats by an excess of dietary calcium intake. In fact, with rats these true digestibility values decreased at the higher levels of calcium intake.

✓ It is evidenced, therefore, that during the period from sexual maturity to maturity the increased loss of endogenous calcium must be due to an increased resorption from the bones themselves, and subsequent excretion. Since there is a decreased exchange rate with age demonstrated (Figure 4.), and little change in absorption from the tract, even when excessive amounts of calcium is supplied, this loss indicates a possible endocrine influence that is augmented with age. It is apparent, therefore, that increasing age during this period of adjustment first affects efficiency of utilization, and then in turn, to a lesser extent, absorption itself.

The rapid equilibrium between the various metabolic pools, and the apparent lability of the reserve calcium in the bone even in old animals would indicate that all or the greater portion of the calcium absorbed is first deposited in bone and subsequently withdrawn for use by the body as needed for the various metabolic processes, including maintenance. This was exemplified further by the increased concentration of labeled calcium in trabecular bones of the pregnant heifers in this study, when apparently the maternal calcium is deposited for subsequent transfer to the fetus (105).✓

The high percentage retention of dietary calcium observed in young growing animals (62, 95, 110) has been interpreted by some investigators to mean that there is no integral requirement of this element for maintenance. However, there was a substantial loss of endogenous calcium at all ages by cattle and rats, and if this loss can be considered as a measure for such requirements (93, 127), it is obvious that even rapidly growing animals have a maintenance requirement for calcium. In the rat this requirement per kilogram of body weight for all ages was more than double that of the bovine, and increased with age.

The dietary calcium needed by animals to make good the endogenous loss will be larger than the losses themselves by an amount that may be calculated from the utilization in the body. Thus for yearling cattle the endogenous loss averaged 6.8 grams per day, but the true digestibility showed an average utilization of forty one per cent, indicating a daily calcium requirement for maintenance of 16.5 grams dietary calcium per day or 2.2 grams per hundred pounds body weight. This apparent high maintenance figure may be due to the high calcium history of these cattle as demonstrated by the increase noted for rats on high calcium, and as indicated by the studies of Hegsted (56) where a low calcium status was shown to give a low dietary endogenous fecal calcium value. In ten day old calves the loss was 0.350 grams, with utilization of ninety three per cent, indicating a requirement of only 0.375 grams dietary calcium per day or 0.48 grams per 100 pounds body weight for maintenance. In like manner, the mature and aged animals would require 2.8 and 4.9 grams per hundred pounds body weight per day respectively. However, the fact that when this dietary calcium was supplied to aged animals, absorption was not increased and negative balance persisted would indicate other adjustments are necessary for utilization.

A study of calcium partition in the gastrointestinal tract indicates that this procedure may serve as a useful index to the net influences of the diversity of mechanisms affecting the calcium concentration in the individual metabolic pools. These values reflect directly or indirectly the behavior of the calcium ions in the animal body and may be a principal determinant for evaluation of animal maintenance and growth requirements for calcium at the different ages.

The distribution pattern of labeled calcium in animals sacrificed after equilibrium can also be used to indicate the behavior of normal calcium ions in the animal body (46, 47, 53, 119). The blood concentration curves show that all calcium 45 ions introduced into the blood reach an immediate equilibrium by exchange and/or deposition in soft tissue and bone. It has been calculated from these curves that every minute about seventy per cent of all the blood calcium is removed (3). However, since the total blood calcium level does not change, this means that the calcium absorbed from the tract or that entering the blood by exchange with the bones replaces about seventy per cent of the blood calcium each minute in the mature animals, and a slightly greater amount in the young of the species (3). The high exchange rate and the rapid equilibrium maintained by the soft tissue with the blood calcium gives a similar pattern except for the short time lag required for equilibrium.

Analytical values showed that soft tissues of cattle or rats contain five to fifteen milligrams of calcium per 100 grams fresh weight, with generally higher values in cartilage, lungs, skin and kidney. These values were not significantly affected by age; and in rats maintained to sacrifice on excess dietary calcium, tissue concentration of ash, calcium and phosphorus were not significantly influenced. Simultaneous radiocalcium distribution studies indicated, however, that there was a tendency toward an accumulation of absorbed calcium 45 in the kidney of rats, but not in cattle, after maturity was reached. Several investigators (72, 73, 83, 118) have reported that ageing is accompanied by an increased concentration of calcium in selected soft tissues under variable experimental conditions. However, whether this is a result of progressive pathology culminating during the period of senescence, or whether it is a so-called normal result or cause of ageing has not been definitely determined. Cornell workers (84) accredited this calcification to an unknown variable in the diet and not to the dietary calcium itself.

Radiocalcium studies with rats and cattle indicate a retardation in disappearance of intravenously administered calcium with increasing age that was reflected by the higher soft tissue concentration values. However, since total calcium levels were not appreciably different in the aged animals, specific activity figures indicated that equilibrium between soft tissue and bone was maintained at a higher level than in the young animal, where exchange rates are higher and the plasma calcium is more rapidly removed by the bones. The lower concentration and exchange rates noted in the livers of old mice by Lansing (75), may have been a reflection of this slower disappearance of radiocalcium from the blood and tissues in the older animals. Likewise, the apparent shift with age of calcium from bone to soft tissue postulated by earlier investigators (58, 73) could be a shift in ratio rather than actual movement of calcium, as was indicated by Copp (26) in his studies with Vitamin D deficient rats. This does not mean, however, that there are no species differences, nor that deviations from normal metabolic functions could not result in changes in this calcium deposition pattern. The ratios of labeled calcium concentration between the various tissues at the different ages further substantiated the fact that, although there were some differences between the several soft tissues, there were no striking effects that could be definitely attributed to age.

In consideration of the facts that calcium 45 in the blood, soft tissues, and bone are in equilibrium, and that practically all of the calcium 45 is eventually deposited in the bone and remains there, it is obvious that in the normal animal after the initial dilution occurs with other metabolic pools, the bone itself determines the rate and the amount of absorbed calcium 45 disappearing from the blood, and ultimately the level at which equilibrium is maintained. The fact that this rate was greater in the young animals (Figure 3.), and that the equilibrium level was higher in the older animals, would mean that the bone of the older animals either had less contact with the plasma or that there was a decreased exchange capacity with age or both. Young bone has been shown, however to have increased vascularity (113, 123), and in vitro studies have demonstrated it to have a higher exchange capacity (26, 103) than bones from older animals.

When animals of different ages, therefore, were sacrificed at the same interval of time after administration, the higher concentration of absorbed radiocalcium in the bones of the ten day old animals showed that these bones had ready access to the calcium in the plasma, and were able to remove it more rapidly than were the bones of older animals. This was indicated further by the decreased concentration with age in the bones themselves and the relatively higher concentration of absorbed calcium 45 in the soft tissue of the older animals. The blood concentration curves for cattle at various ages reflected this trend and substantiated these findings. In the young calf for example, seven days after administration the specific activity values for bone and soft tissues were essentially the same, while in the older cattle these ratios spread until in the aged animal there was many times the concentration of calcium 45 per unit of total calcium in the soft tissues as in bone. This trend was similar for rats but because of the relatively higher dose per unit of weight administered, the specific activity values were higher at all ages than that noted for cattle.

It is known (23, 59) that exchange is an important factor in the uptake of radiocalcium by the skeleton, and there is evidence that it is perhaps the major factor in the mature and aged animals. However, in the young animal exchange is not the only mechanism. The plasma apparently has ready access to the bone crystals and the radiocalcium is removed from the plasma very rapidly, with fixation and very slow loss, indicating that it may be incorporated into the bone salts themselves (26). Whereas, in the mature and aged animal the plasma not only has less access to the bone organic matrix but there is a decreased amount of new bone salts being formed. This resulting slower removal from the plasma then may be interpreted to mean that exchange alone is taking place with the calcium present in the bone salts, with subsequent decreased deposition (Figure 5.).

It is apparent, however, that there is a wide difference between bones and areas of the same bone in the animal body. For instance bone immediately adjacent to the Haversian canals has demonstrated a greater affinity for calcium than that from areas more remote from the vascular system (1), and trabecular bone has been shown to absorb more calcium 45 and have a higher turnover rate than compact bone (34, 51). Analytical and radiocalcium concentration values for selected bones from rats and cattle show these variations, which have been interpreted to indicate a difference in metabolic activity (51, 52) that decreases with increased age. It is further demonstrated that calcium deposition is governed not only by the access of the plasma calcium to the bone, but by the specific characteristic of the bone itself, both of which appear to be influenced by age.

SUMMARY

Chemical and radioisotope procedures were used concurrently in studies with forty five cattle and two hundred albino rats to investigate the effects of age upon calcium absorption, deposition and exchange in the animal body.

Calcium absorption and utilization efficiency were highest in young animals, decreased rapidly with age to sexual maturity, then more slowly to maturity, and again decreased at senescence. The endogenous fecal calcium increased with age, being ten, twenty and twenty five milligrams per kilogram body weight for cattle ten days, six months and twelve years old respectively. Values for rats of corresponding ages were two to four times higher than those for cattle.

Deposition of calcium in selected tissues was indicated by chemical analysis and calcium 45 concentration values in animals sacrificed after equilibrium had been reached. No age effects were demonstrated in the per cent of calcium, phosphorus and ash in the soft tissues. However, in bone these values increased rapidly with age to sexual maturity and then less rapidly to maturity and senescence. Concentration of absorbed radiocalcium in soft tissues increased with age of animal being twenty to thirty times higher in the aged animals than in those ten days old. This was interpreted as indicating a lower uptake and a slower turnover rate by the bones of the older animals. Bone autoradiograms and bone concentration data substantiated these findings, indicating reduced vascularity and exchange rate with increasing age. Calcium deposition appeared to be dependent upon the access of bone to plasma calcium and

the specific characteristic of the bone itself, both of which were affected by age.

The exchange capacity and metabolic activity of selected tissue in cattle and rats at the various ages were demonstrated by specific activity values. These values were higher for soft tissue than for bone, and higher for trabecular than cortical bone. Bones from young animals demonstrated a higher value than did those from older animals, indicating an increased vascularity and affinity for circulating calcium. The ratios of the specific activities of selected tissues were employed to estimate relative trends in rate and quantity of calcium 45 laydown as a function of the total calcium present. The spread in the ratios of soft tissue compared to bone with increasing age further demonstrated the decreased exchange rate in bones of the older animals. In the young animal both exchange and fixation by the bone salts themselves were indicated, whereas in the bone of mature and aged animal exchange appeared to be the major factor for calcium 45 uptake.

A simple isotope dilution method employing concentration values of absorbed radiocalcium in selected trabecular and cortical bone has been presented for the estimation of skeletal mass in rats and cattle. Application and limitations are discussed and values for rats at the various ages were tabulated.

Calcium absorption, deposition or exchange were not significantly affected in rats maintained to sacrifice on an excess level of dietary calcium. However, pregnancy in heifers appeared to increase in some way the capacity for calcium 45 storage or retention.

The behavior of labeled calcium in tissues of animals, sacrificed after equilibrium had been reached, was shown to indicate indirectly the behavior of stable calcium ions in the body of the rat and bovine. When accompanied by simultaneous chemical determinations, this sensitive relationship offers a basis for a fuller comprehension of the ageing processes that may conceivably lead to a prophylactic measure which would delay the onset of senescence.

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BIOGRAPHICAL ITEMS

The author was born in Knox County, Tennessee June 5, 1914. He attended the county public schools and was graduated as class valedictorian from Halls High School in 1932. He was graduated from the University of Tennessee with a Bachelor of Science degree in 1936, and with a Master of Science degree in Agriculture in 1937. Following a years service with the Tennessee Agricultural Extension Service, he accepted an Assistantship with the Animal Husbandry Department at Ohio State University in 1939. He terminated his studies there with a Master of Science degree in 1941 to enlist in the United States Army. After seven months, he was commissioned a First Lieutenant in the Medical Service Corps and served four years in the United States and the Southwest Pacific Area as Nutrition Consultant to Post and Base Surgeons. He was discharged in 1946 as Captain, and accepted an Instructorship with the Animal Husbandry Staff, University of Tennessee. In 1947, he was made Assistant, and in 1948 Associate Professor of Animal Husbandry. In 1951 he was made Senior Scientist in charge of the nutrition and metabolism studies at the Oak Ridge Station. He entered the graduate school at the University of Florida in the Fall of 1951, and completed the requirements for the Doctor of Philosophy degree in Animal Nutrition in June 1953. He is a member of Alpha Zeta, Phi Kappa Phi, Gamma Sigma Delta, Sigma Xi, Phi Sigma, Omicron Delta Kappa, American Society of Animal Production, American Chemical Society, American Dairy Science Association, and various professional and civic clubs.

This dissertation was prepared under the direction of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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